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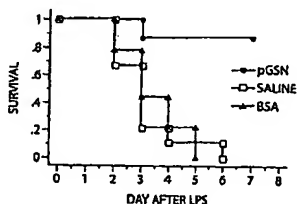
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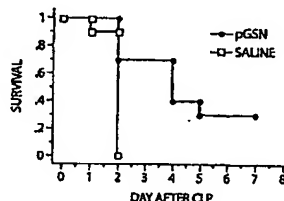
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(54) Title: **USE OF GELSOLIN TO TREAT INFECTIONS**



A



B

(57) Abstract: The invention relates to the use of gelsolin to treat infections and to monitor the treatment of infections. The invention also provides methods up-regulating interleukin expression and methods for down-regulating pro-inflammatory cytokine expression.

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## USE OF GELSOLIN TO TREAT INFECTIONS

### FIELD OF THE INVENTION

The invention is directed to the use of gelsolin to treat infections and sepsis and  
5 to the use of gelsolin to monitor and evaluate treatments of infections. The invention  
also provides methods for up-regulating interleukin expression and methods for down-  
regulating pro-inflammatory cytokine expression.

### BACKGROUND OF THE INVENTION

10 Despite significant advances in diagnosis and therapy, infections and sepsis  
remain a major cause of morbidity and mortality throughout the world. Even with  
aggressive management, many patients with severe infections develop complications and  
some die. Sepsis claims more than 200,000 lives in the United States annually (Angus,  
D. C. & Wax, R. S. (2001) *Crit Care Med* 29, S109-16). An intense inflammatory  
15 response accompanies the early phase of sepsis with markedly increased plasma levels of  
pro-inflammatory cytokines (Riedemann, N. C., Guo, R. F. & Ward, P. A. (2003) *Nat*  
*Med* 29, 517-24), in addition to other biochemical abnormalities. Much concerted  
research has gone into attempts at inhibition of specific inflammatory mediators in hope  
of developing pharmacologic treatments for sepsis. Nevertheless, activated protein C  
20 (APC) is the only drug proven to reduce the mortality of severe sepsis with an absolute  
reduction of death by 6% (Bernard, G. R., Vincent, J. L., Laterre, P. F., LaRosa, S. P.,  
Dhainaut, J. F., Lopez-Rodriguez, A., Steingrub, J. S., Garber, G. E., Helterbrand, J. D.,  
Ely, E. W. & Fisher, C. J., Jr. (2001) *N Engl J Med* 344, 699-709).

The negative health effects of infections and sepsis provide a strong incentive to  
25 identify new treatments as well as improved tests and approaches to evaluate therapy of  
infections and sepsis.

### SUMMARY OF THE INVENTION

This invention is based on the surprising discovery that gelsolin treats infections  
30 and protects against the toxic manifestations of infections. We have found that mice  
injected with gelsolin subsequent to the exposure to an infectious agent unexpectedly  
survived better than mice given saline. Thus, the invention involves in one aspect, the

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administration of gelsolin to a subject to treat an infection. The invention is also directed to methods of using gelsolin to treat the biological effects of infections.

Gelsolin (GSN), specifically cytoplasmic gelsolin (cGSN), first discovered as an intracellular actin-binding protein involved in cell motility (Yin, H. L. & Stossel, T. P. (1979) *Nature* 281, 583-6) is also an abundant secretory protein (Yin, H. L., Kwiatkowski, D. J., Mole, J. E. & Cole, F. S. (1984) *J Biol Chem* 259, 5271-6). The exported isoform of gelsolin, designated plasma gelsolin (pGSN), has 25 additional amino acids and originates from alternative splicing of a single gene (Kwiatkowski, D. J., Stossel, T. P., Orkin, S. H., Mole, J. E., Colten, H. R. & Yin, H. L. (1986) *Nature* 323, 455-8).

In each of the following aspects and embodiments of the invention, the use of pGSN is preferred.

According to one aspect of the invention, methods of treating an infection in a subject are provided. The methods include administering gelsolin to the subject having or at risk of having an infection in an effective amount to treat the infection. The infection may be caused by a gram-positive bacterium, an acid-fast bacillus, a spirochete, an actinomycete, a virus, a fungus, a parasite, *Ureaplasma* species including *Ureaplasma urealyticum*, *Mycoplasma* species including *Mycoplasma pneumonia*, *Rickettsia* species, *Chlamydia* species including *Chlamydia psittaci*, *Chlamydia trachomatis* and *Chlamydia pneumoniae*, and *Pneumocystis* species including *Pneumocystis carinii*. In some embodiments, the subject is otherwise free of indications calling for treatment with gelsolin. In some embodiments, the gelsolin is administered subsequent to exposure of the subject to the infection. In certain embodiments, the gelsolin is administered at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours subsequent to exposure of the subject to the infection. In some embodiments, the gelsolin is administered at least about 1, 2, 3, 4, 5, 6, 7, or more days subsequent to exposure of the subject to the infection.

According to yet another aspect of the invention, methods of treating a gram-negative bacterial infection in a subject are provided. The methods include administering gelsolin to the subject in an effective amount at a time subsequent to the exposure of the subject to a gram-negative bacterial infection. In some embodiments, the subject is otherwise free of indications calling for treatment with gelsolin. In some

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embodiments, the gelsolin is administered at least about one hour subsequent to exposure of the subject to the gram-negative bacterial infection. In certain embodiments, the gelsolin is administered at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours subsequent to after exposure of the subject to the  
5 gram-negative bacterial infection. In some embodiments, the gelsolin is administered at least about 1, 2, 3, 4, 5, 6, 7, or more days subsequent to exposure of the subject to the gram-negative bacterial infection. In some embodiments, gelsolin is administered prior to exposure of the subject to the gram-negative bacterial infection.

According to another aspect of the invention, methods of treating or preventing  
10 the effects of lipopolysaccharide endotoxin (LPS) in a subject are provided. The methods include administering to the subject an effective amount of gelsolin at a time subsequent to LPS exposure to protect the subject against the effects of LPS. In some embodiments, the subject is otherwise free of indications calling for treatment with gelsolin. In some embodiments, the gelsolin is administered at least about one hour  
15 subsequent to the exposure of the subject to the LPS. In certain embodiments, the gelsolin is administered at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 or more hours subsequent to exposure of the subject to LPS. In some embodiments, the gelsolin is administered at least about 1, 2, 3, 4, 5, 6, 7, or more days subsequent to exposure of the subject to LPS.

According to another aspect of the invention, methods of treating or preventing  
20 gram-negative bacterial septic shock in a subject exposed to LPS are provided. The methods include administering to the subject an effective amount of gelsolin at a time subsequent to LPS exposure to treat the gram-negative bacterial septic shock in the subject. In some embodiments, the subject is free of indications calling for treatment  
25 with gelsolin. In some embodiments, the gelsolin is administered at least about one hour subsequent to exposure of the subject to LPS. In certain embodiments, the gelsolin is administered at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 hours subsequent to exposure of the subject to LPS. In some  
30 embodiments, the gelsolin is administered at least about 1, 2, 3, 4, 5, 6, 7, or more days subsequent to exposure of the subject to LPS.

According to another aspect of the invention, a method for up-regulating interleukin (IL) expression in a subject is provided. The method involves administering

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gelsolin to the subject in an effective amount to up-regulate IL expression in the subject. In some embodiments, the subject is free of indications calling for treatment with gelsolin. Up-regulation of IL expression may be either due to increased expression of the IL or due to decreased degradation of the IL or a combination of an increased  
5 expression of the IL and a decreased degradation of the IL.

In some embodiments, the expression of IL is increased by at least approximately 25% relative to control. In other embodiments, the expression of IL is increased by at least approximately 50% or 75% relative to control. In some embodiments, the expression of IL is increased by at least approximately 2-fold relative to control. In  
10 some embodiments, the IL expression is increased at least approximately 3-fold, 5-fold, 10-fold, 50-fold, 100-fold, 200-fold, 500-fold, or 1000-fold relative to control. In general, the IL expression level in a control is the level of IL expression in a subject to whom gelsolin was not administered but is otherwise identical to the treated subject. Methods of measuring IL levels are known to those of ordinary skill in the art.

15 According to another aspect of the invention, a method for up-regulating IL expression *in vitro* is provided. The method involves contacting a cell capable of expressing IL with a sufficient amount of gelsolin to up-regulate the level of IL expression in the cell. In some embodiments, the expression of IL is increased by at least approximately 25% relative to control. In other embodiments, the expression of IL  
20 is increased by at least approximately 50% or 75% relative to control. In some embodiments, the expression of IL is increased by at least approximately 2-fold relative to control. In general, the level of IL expression in a control is that level of expression in a cell that is not contacted with gelsolin but is otherwise identically treated to the cell contacted with gelsolin. In some embodiments, the IL expression is increased at least  
25 approximately 3-fold, 5-fold, 10-fold, 50-fold, 100-fold, 200-fold, 500-fold, or 1000-fold relative to control. Methods of measuring IL levels are known to those of ordinary skill in the art.

The time period in which the IL expression is increased is, at least in part, a function of the cell type and on the specific culture vessel used. In general, this time  
30 period ranges from 2-3 hours (for short-term increase) to about 2-3 days (for medium-term increase) to several weeks (for long-term increase). Routine procedures known to those of ordinary skill in the art can be used to determine the level of IL expression as a

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function increasing doses of gelsolin or increasing incubation time or of the cells with gelsolin. One preferred IL is IL-10.

According to another aspect of the invention, a method for down-regulating pro-inflammatory cytokine expression in a subject is provided. The method involves  
5 administering to the subject gelsolin in an effective amount to down-regulate pro-inflammatory cytokine expression in the subject. In some embodiments, the subject is otherwise free of indications calling for treatment with gelsolin. Down-regulation of pro-inflammatory cytokine expression may be either due to a decreased expression of the cytokine or due to an increased degradation of the cytokine or a combination of a  
10 decreased expression of the cytokine and an increased degradation of the cytokine. In general, the expression of pro-inflammatory cytokine is decreased by at least approximately 10% relative to control. In some embodiments, the expression of pro-inflammatory cytokine is decreased by at least approximately 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% relative to control. In some embodiments the  
15 expression of pro-inflammatory cytokine is decreased by 100% relative to control. In general, the pro-inflammatory cytokine expression level in a control is the level of pro-inflammatory cytokine expression in a subject to whom gelsolin was not administered but is otherwise identical to the treated subject. Methods of measuring pro-inflammatory cytokine levels are known to those of ordinary skill in the art. One preferred pro-  
20 inflammatory cytokine is IL-1 $\beta$ . Another preferred pro-inflammatory cytokine is IFN- $\alpha$ .

According to one aspect of the invention, a method for down-regulating expression of pro-inflammatory cytokines *in vitro* is provided. The method involves contacting a cell capable of expressing a pro-inflammatory cytokine with a sufficient amount of gelsolin to down-regulate the level of pro-inflammatory cytokine expression  
25 in the cell. In general, the expression of a pro-inflammatory cytokine is decreased by at least approximately 10% relative to control. In general, the level of a pro-inflammatory cytokine expression in a control is that level of expression in a cell that is not contacted with gelsolin but is otherwise identically treated to the cell contacted with gelsolin. In some embodiments, the pro-inflammatory cytokine expression is decreased at least  
30 approximately 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 99% relative to control. In some embodiments the expression of pro-inflammatory cytokine is decreased

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by 100% relative to control. Methods of measuring pro-inflammatory cytokine levels are known to those of ordinary skill in the art.

The time period in which the pro-inflammatory cytokine expression is decreased is, at least in part, a function of the cell type and on the specific culture vessel used. In general, this time period ranges from 2-3 hours (for short-term decrease) to about 2-3 days (for medium-term decrease) to several weeks (for long-term decrease). Routine procedures known to those of ordinary skill in the art can be used to determine the level of pro-inflammatory cytokine expression as a function of increasing doses of gelsolin or increasing incubation time of the cells with the gelsolin.

According to another aspect of the invention, a method for treating a subject to reduce the risk of an infection is provided. The method comprises selecting a subject on the basis that the subject is known to have a below-normal level of gelsolin and administering to the subject an agent for reducing the risk of an infection in an amount effective to lower the subject's risk of developing an infection. The agent may be gelsolin and/or an anti-infective agent. In some embodiments, the subject is otherwise free of indications calling for treatment with gelsolin.

A "below-normal level of gelsolin" is a gelsolin level is at least 10% less than the measured mean level for a given population of subjects. The mean gelsolin level can depend upon the particular population of subjects. For example, an apparently healthy population will have a different "normal" range of gelsolin than will a population of subjects which have had a prior infection or other condition. In some embodiments, the gelsolin level is at least 10% less than the measured mean level for a given population of subjects. In other embodiments, the gelsolin level is at least 20% less than the measured mean level for a given population of subjects. In still other embodiments, the gelsolin level is at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99% or 100% less than the measured mean level for a given population of subjects. In one of the preferred embodiments, the gelsolin level is below about 2.4  $\mu\text{M/L}$  (micromoles/Liter) of plasma.

In some embodiments the subject is otherwise free of indications calling for treatment with the agent. When the agent is an anti-infective agent, a subject free of indications calling for treatment with an anti-infective agent is a subject who has no signs or symptoms of an infection. Signs and symptoms of an infection are well known to those of ordinary skill in the art. Gelsolin is indicated for the treatment of actin-

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related disorders such as Adult Respiratory Distress Syndrome (ARDS), fulminant hepatic necrosis, acute renal failure, muscle injury, disorders characterized by elevated levels of BUN and/or creatinine. Actin-related disorders are known to those of ordinary skill in the art.

5           In other embodiments, the subject is apparently healthy. As used herein an "apparently healthy subject" is a subject who has no signs and/or symptoms of a disease.

The infection may be caused by one or more of a number of organisms such as a bacterium, a virus, a fungus, or a parasite.

10           The anti-infective agent may be an anti-bacterial, an anti-viral, and anti-fungal, or an anti-parasitic agent.

Examples of anti-bacterial agents, anti-viral agents, anti-fungal agents, and anti-parasitic agents are listed below.

15           The gelsolin may be administered orally, sublingually, buccally, intranasally, intravenously, intramuscularly, intrathecally, intraperitoneally, subcutaneously, intradermally, topically, rectally, vaginally, intrasynovially, or intravitreally.

20           According to another aspect of the invention, a method for evaluating the efficacy of a therapy with a therapeutic agent other than a gelsolin for treating or preventing an infection is provided. The method involves obtaining a level of a gelsolin in a subject undergoing the therapy to treat or prevent an infection. The level of the gelsolin is compared to a predetermined value corresponding to a control level of gelsolin (e.g., in an apparently healthy population). A determination of whether the level of the gelsolin is below the predetermined level is indicative of whether the therapy is efficacious. In some embodiments, obtaining a level of the gelsolin is repeated so as to monitor the human subject's level of the gelsolin over time.

25           According to yet another aspect of the invention method for deciding on the course of a therapy in a subject is provided. The method involves obtaining a level of gelsolin in a subject undergoing a therapy to treat an infection. The level of gelsolin is compared to a predetermined value corresponding to a control level of gelsolin (e.g., in an apparently healthy population). Whether the level of gelsolin obtained is above or below the predetermined level is determined and the course of the therapy is decided based on such determination. In some embodiments, obtaining a level of gelsolin is repeated so as to monitor the subject's level of gelsolin over time.

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According to yet another aspect of the invention, a method for treating a subject with a decreased level of gelsolin is provided. The method involves treating the subject with a first therapy for treating an infection. A level of gelsolin in the subject is obtained. The level of gelsolin is compared to a predetermined value corresponding to a control level of gelsolin (e.g., in an apparently healthy population). If the predetermined level of gelsolin is not reached, the subject is treated with a second therapy for treating an infection and the level of the gelsolin is measured and compared to the predetermined level of the gelsolin until the predetermined level of the gelsolin is reached.

According to still yet another aspect of the invention, a method for characterizing an subject's risk profile of developing a future infection is provided. The method comprises obtaining a level of gelsolin in the subject and comparing the level of the marker to a predetermined value. The subject's risk profile of developing an infection is characterized based upon the level of gelsolin in comparison to the predetermined value. A level of gelsolin below the predetermined level is indicative that the subject is at an elevated risk of developing an infection and a level of gelsolin above the predetermined level is indicative that the subject is not at an elevated risk of developing an infection.

In some embodiments, the predetermined value is about 2.4  $\mu\text{M/L}$  of plasma or lower.

In some embodiments, the subject is an apparently healthy subject.

Each of the limitations of the invention can encompass various embodiments of the invention. It is, therefore, anticipated that each of the limitations of the invention involving any one element or combinations of elements can be included in each aspect of the invention. The invention is capable of other embodiments and of being practiced or of being carried out in various ways. Also, the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of "including," "comprising," or "having," "containing", "involving", and variations thereof herein, is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

These and other aspects of the invention will be described in more detail below in connection with the detailed description of the invention.

All documents identified in this application are incorporated in their entirety herein by reference.

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### BRIEF DESCRIPTION OF DRAWINGS

The figures are illustrative only and are not required for enablement of the invention disclosed herein.

5        Figure 1 is a graph of plasma gelsolin levels in septic mice. (A) Mice were injected with increasing doses of non-lethal LPS intraperitoneally (IP) and then bled 24 hours later. pGSN level inversely correlated with LPS doses ( $P < 0.05$ , Spearman Correlation). Conversely, albumin was unchanged. (B) Mice were subjected to CLP (Cecal Ligation and Puncture) or no surgery and plasma collected 24 hours later. The  
10    left graph shows that pGSN levels of mice subjected to CLP were significantly lower than control mice ( $P < 0.001$ ) while the right graph shows plasma albumin levels were actually higher in CLP mice ( $P = 0.02$ ).

      Figure 2 is a plot of survival in septic mice. (A) In mice challenged with lethal LPS, those treated with pGSN had significantly better survival compared to BSA  
15    ( $P < 0.001$ ) or saline treated mice ( $P < 0.001$ ). (B) Mice subjected to CLP had similar favorable response to pGSN and had much better survival ( $P = 0.001$ ).

      Figure 3 is a graph of the pGSN levels in lethal endotoxemic mice treated with or without exogenous pGSN. Open bars denote mice received saline treatment and solid  
20    bars denote mice received exogenous pGSN treatment. Endogenous pGSN levels dropped to near 50% of normal within 6 hours of lethal LPS challenge and persisted for at least 24 hours ( $*P < 0.015$ , compared with unchallenged mice). Administration of exogenous pGSN at the time of LPS challenge successfully raised pGSN levels ( $**P < 0.021$ , comparing pGSN treated and untreated mice within the same group).

      Figure 4 is a graph of the cytokine profiles of endotoxemic mice treated with or  
25    without pGSN at 6 hr and 24 hr after LPS (y-axis in log scale). (A) Cytokine profiles did not differ between pGSN treated (solid bars) and untreated (open bars) mice 6 hours after LPS challenge. (B) However, 24 hours after LPS, saline-treated mice had significantly higher levels of GM-CSF, IFN- $\gamma$ , and IL-1 $\beta$  ( $P < 0.03$  for all) by as much as 10 folds compared to pGSN-treated mice. In contrast, IL-10 level was significantly higher in  
30    pGSN-treated mice ( $P < 0.03$ ).

      Figure 5 is a graph of plasma gelsolin levels in *E. coli* LPS-challenged and unchallenged C3H/HeJ mice. LPS had no effect on pGSN levels of TLR4 mutants.

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C3H/HeJ mice injected with LPS that was lethal to C57BL/6 mice did not exhibit any signs of illness and had unaltered pGSN levels.

Figure 6 is a Western blot analysis, staining for pGSN, on tissue extracts of mice challenged with or without LPS. The blot shows that lung has the highest concentration of pGSN comparing to skeletal muscle, heart, kidney, and liver in both normal and endotoxemic mice.

Figure 7 is a plot comparing binding to LPS of pGSN vs BSA. Fluorescent-based binding study of pGSN and LPS showing a classic binding curve of fluorescent LPS plateauing at 250  $\mu$ g/well of pGSN. BSA, serving as the control, protein showed minimal affinity to LPS.

Figure 8 is a graph of TNF- $\alpha$  levels of media from THP-1 cells treated without LPS (unstimulated), LPS only, pGSN only, LPS and pGSN, LPS and BSA, and BSA only. LPS stimulated THP-1 cells treated with pGSN or BSA had similar levels of TNF- $\alpha$  ( $P>0.05$ ).

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention is based, in part, on the discovery that the administration of gelsolin protects a subject from infection. Thus, the invention includes, in some aspects, administering gelsolin to a subject for the treatment of infection in the subject. We have discovered that gelsolin antagonizes the toxic effects lipopolysaccharide endotoxin (LPS), the cell wall material of gram-negative bacteria known to be responsible for many of the manifestations of the gram-negative bacterial infection.

We have also discovered that the administration of gelsolin to a subject following exposure of the subject to an infection can treat an infection and can reduce or prevent the toxic effects of the infection in the subject. Preferably, the treatment of an infection involves treatment of the signs and symptoms of the infection.

The term "treatment" or "treating" is intended to include prophylaxis, amelioration, prevention or cure of infections.

As used herein the term "subject" means any mammal that may be in need of treatment. Subjects include but are not limited to: humans, non-human primates, cats, dogs, sheep, pigs, horses, cows, rodents such as mice, hamsters, and rats. Preferred subjects are human subjects.

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As used herein the term "gelsolin" encompasses wild type gelsolin (GenBank accession No.: X04412), isoforms, analogs, variants, fragments or functional derivatives of gelsolin. Gelsolin encompasses native as well as synthetic and recombinant gelsolin and gelsolin analogs. Gelsolin, specifically cGSN, is an abundant secretory protein (Yin, H. L., Kwiatkowski, D. J., Mole, J. E. & Cole, F. S. (1984) *J Biol Chem* 259, 5271-6). The exported isoform of gelsolin, pGSN, has 25 additional amino acids and originates from alternative splicing of a single gene (Kwiatkowski, D. J., Stossel, T. P., Orkin, S. H., Mole, J. E., Colten, H. R. & Yin, H. L. (1986) *Nature* 323, 455-8). In the different aspects and embodiments of the invention, the use of pGSN is preferred.

A "gelsolin analog" refers to a compound substantially similar in function to either the native gelsolin or to a fragment thereof. Gelsolin analogs include biologically active amino acid sequences substantially similar to the gelsolin sequences and may have substituted, deleted, elongated, replaced, or otherwise modified sequences that possess bioactivity substantially similar to that of gelsolin. For example, an analog of gelsolin is one which does not have the same amino acid sequence as gelsolin but which is sufficiently homologous to gelsolin so as to retain the bioactivity of gelsolin. Bioactivity can be determined, for example, by determining the properties of the gelsolin analog and/or by determining the ability of the gelsolin analog to reduce or prevent the effects of an infection. One example of gelsolin bioactivity assay is gelsolin's ability to stimulate actin nucleation. Gelsolin bioactivity assays are described in the Example and are known to those of ordinary skill in the art.

A "fragment" is meant to include any portion of a gelsolin molecule which provides a segment of gelsolin which maintains the bioactivity of gelsolin; the term is meant to include gelsolin fragments which are made from any source, such as, for example, from naturally-occurring peptide sequences, synthetic or chemically-synthesized peptide sequences, and genetically engineered peptide sequences.

A "variant" of gelsolin is meant to refer to a compound substantially similar in structure and bioactivity either to native gelsolin, or to a fragment thereof.

A "functional derivative" of gelsolin is a derivative which possesses a bioactivity that is substantially similar to the bioactivity of gelsolin. By "substantially similar" is meant activity which is quantitatively different but qualitatively the same. For example, a functional derivative of gelsolin could contain the same amino acid backbone as

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gelsolin but also contains other modifications such as post-translational modifications such as, for example, bound phospholipids, or covalently linked carbohydrate, depending on the necessity of such modifications for the performance of the diagnostic assay or therapeutic treatment. As used herein, the term is also meant to include a chemical derivative of gelsolin. Such derivatives may improve gelsolin's solubility, absorption, biological half life, etc. The derivatives may also decrease the toxicity of gelsolin, or eliminate or attenuate any undesirable side effect of gelsolin, etc. Derivatives and specifically, chemical moieties capable of mediating such effects are disclosed in Remington's Pharmaceutical Sciences (1980). Procedures for coupling such moieties to a molecule such as gelsolin are well known in the art. The term "functional derivative" is intended to include the "fragments," "variants," "analogues," or "chemical derivatives" of gelsolin.

The invention involves in some aspects, methods for treating infection in a subject. The methods involve administering gelsolin to a subject for treating the infection. The subject is known to have, is suspected of having been exposed, or is at risk of being exposed, or who has been exposed to an infection. The gelsolin is administered in an amount effective to treat the infection in the subject.

A response to a treatment method of the invention can, for example, be measured by determining the physiological effects of the treatment, such as the decrease or lack of symptoms following administration of the treatment.

An "infection" or "infectious disease", as used herein, refers to a disorder arising from the invasion of a host, superficially, locally, or systemically, by an infectious organism. Infectious organisms include bacteria, viruses, parasites, fungi, and protozoa.

Bacteria include gram-negative and gram-positive bacteria. Examples of gram-positive bacteria include Pasteurella species, Staphylococcus species including Staphylococcus aureus, Streptococcus species including Streptococcus pyogenes group A, Streptococcus viridans group, Streptococcus agalactiae group B, Streptococcus bovis, Streptococcus anaerobic species, Streptococcus pneumoniae, and Streptococcus faecalis, Bacillus species including Bacillus anthracis, Corynebacterium species including Corynebacterium diphtheriae, aerobic Corynebacterium species, and anaerobic Corynebacterium species, Diphtheroids species, Listeria species including Listeria monocytogenes, Erysipelothrix species including Erysipelothrix rhusiopathiae,

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Clostridium species including Clostridium perfringens, Clostridium tetani, and Clostridium difficile.

Gram-negative bacteria include Neisseria species including Neisseria gonorrhoeae and Neisseria meningitidis, Branhamella species including Branhamella catarrhalis, Escherichia species including Escherichia coli, Enterobacter species, Proteus species including Proteus mirabilis, Pseudomonas species including Pseudomonas aeruginosa, Pseudomonas mallei, and Pseudomonas pseudomallei, Klebsiella species including Klebsiella pneumoniae, Salmonella species, Shigella species, Serratia species, Acinetobacter species; Haemophilus species including Haemophilus influenzae and Haemophilus ducreyi, Brucella species, Yersinia species including Yersinia pestis and Yersinia enterocolitica, Francisella species including Francisella tularensis, Pasturella species including Pasteurella multocida, Vibrio cholerae, Flavobacterium species, meningosepticum, Campylobacter species including Campylobacter jejuni, Bacteroides species (oral, pharyngeal) including Bacteroides fragilis, Fusobacterium species including Fusobacterium nucleatum, Calymmatobacterium granulomatis, Streptobacillus species including Streptobacillus moniliformis, Legionella species including Legionella pneumophila.

Other types of bacteria include acid-fast bacilli, spirochetes, and actinomycetes.

Examples of acid-fast bacilli include Mycobacterium species including Mycobacterium tuberculosis and Mycobacterium leprae.

Examples of spirochetes include Treponema species including Treponema pallidum, Treponema pertenue, Borrelia species including Borrelia burgdorferi (Lyme disease), and Borrelia recurrentis, and Leptospira species.

Examples of actinomycetes include: Actinomyces species including Actinomyces israelii, and Nocardia species including Nocardia asteroides.

Examples of viruses include but are not limited to: Retroviruses, human immunodeficiency viruses including HIV-1, HDTV-III, LAVE, HTLV-III/LAV, HIV-III, HIV-LP, Cytomegaloviruses (CMV), Picornaviruses, polio viruses, hepatitis A virus, enteroviruses, human Coxsackie viruses, rhinoviruses, echoviruses, Calciviruses, Togaviruses, equine encephalitis viruses, rubella viruses, Flaviruses, dengue viruses, encephalitis viruses, yellow fever viruses, Coronaviruses, Rhabdoviruses, vesicular stomatitis viruses, rabies viruses, Filoviruses, ebola virus, Paramyxoviruses,

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parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus (RSV), Orthomyxoviruses, influenza viruses, Bunyaviruses, Hantaan viruses, phleboviruses and Nairo viruses, Arena viruses, hemorrhagic fever viruses, reoviruses, orbiviruses, rotaviruses, Birnaviruses, Hepadnaviruses, Hepatitis B virus, parvoviruses, Papovaviridae, papilloma viruses, polyoma viruses, Adenoviruses, Herpesviruses including herpes simplex virus 1 and 2, varicella zoster virus, Poxviruses, variola viruses, vaccinia viruses, Iridoviruses, African swine fever virus, delta hepatitis virus, non-A, non-B hepatitis virus, Hepatitis C, Norwalk viruses, astroviruses, and unclassified viruses.

- 10 Examples of fungi include, but are not limited to: Cryptococcus species including Cryptococcus neoformans, Histoplasma species including Histoplasma capsulatum, Coccidioides species including Coccidioides immitis, Paracoccidioides species including Paracoccidioides brasiliensis, Blastomyces species including Blastomyces dermatitidis, Chlamydia species including Chlamydia trachomatis, Candida species including Candida  
15 albicans, Sporothrix species including Sporothrix schenckii, Aspergillus species, and fungi of mucormycosis.

- Other infectious organisms include parasites. Parasites include Plasmodium species, such as Plasmodium species including Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, and Plasmodium vivax and Toxoplasma gondii. Blood-  
20 borne and/or tissues parasites include Plasmodium species, Babesia species including babesia microti and Babesia divergens, Leishmania species including Leishmania tropica, Leishmania species, Leishmania braziliensis, Leishmania donovani, Trypanosoma species including Trypanosoma gambiense, Trypanosoma rhodesiense (African sleeping sickness), and Trypanosoma cruzi (Chagas' disease).

- 25 Other medically relevant microorganisms have been described extensively in the literature, e.g., see C.G.A Thomas, *Medical Microbiology*, Bailliere Tindall, Great Britain 1983, the entire contents of which is hereby incorporated by reference.

- In another aspect of the invention, a method for up-regulating interleukin (IL) expression in a subject is provided. The method comprises administering to the subject  
30 gelsolin in an effective amount to up-regulate IL expression. Up-regulation of IL may be measured by determining the physiological effects of the IL following administration of gelsolin. Physiological effects of IL are known to those of ordinary skill in the art.

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In another aspect of the invention, a method for down-regulating a pro-inflammatory cytokine expression in a subject is provided. The method comprises administering gelsolin to the subject in an effective amount to down-regulate pro-inflammatory cytokine expression. Down-regulation of pro-inflammatory cytokines may be measured by determining the physiological effects of pro-inflammatory cytokines following administration of gelsolin. Physiological effects of pro-inflammatory cytokines are known to those of ordinary skill in the art.

Other assays are known to one of ordinary skill in the art and can be employed for measuring the level of the response.

In another aspect of the invention, a method for monitoring a subject is provided. The method involves obtaining a level of gelsolin in a subject undergoing therapy to treat an infection. The level of gelsolin is compared to a predetermined value corresponding to a control level of gelsolin (e.g., in an apparently healthy population). A determination of whether the level of gelsolin is below a predetermined level is indicative of whether the subject would benefit from continued therapy with the same therapy or would benefit from a change in therapy. In some embodiments, obtaining a level of gelsolin is repeated so as to monitor the subject's levels of gelsolin over time. In some embodiments, the subject may have been undergoing the therapy for at least 1, 2, 3, 4, 5, 6, 7 or more days. In some embodiments, the subject may have been undergoing the therapy for at least 1, 2, 3, 4 or more weeks.

A change in therapy with gelsolin refers to an increase in the dose of the gelsolin, a switch from gelsolin to another agent, the addition of another agent to the gelsolin therapeutic regimen, or a combination thereof.

According to another aspect of the invention, a method for evaluating the efficacy of a therapy for treating or reducing the risk of an infection is provided. The method involves obtaining a level of gelsolin in a subject undergoing therapy to treat or prevent an infection. The level of gelsolin is compared to a predetermined value corresponding to a control level of gelsolin (e.g., in an apparently healthy population). A determination of whether the level of gelsolin is below a predetermined level is indicative of whether the therapy is efficacious. In some embodiments, the subject may have been undergoing the therapy for at least 1, 2, 3, 4, 5, 6, 7 or more days. In some embodiments, the human subject may have been undergoing the therapy for at least 1, 2, 3, 4 or more weeks.



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One aspect of the invention is directed to the measurement of gelsolin to guide treatments in order to improve outcome in subjects. On-therapy levels of gelsolin have predictive value for response to treatments of infections or sepsis. The on-therapy levels of gelsolin are additive to prior art predictors of outcome in infections.

5           Subjects who would benefit from this aspect of this invention are subjects who are undergoing therapy to treat or prevent an infection (i.e., a subject "on-therapy"). A subject on-therapy is a subject who already has been diagnosed and is in the course of treatment with a therapy for treating an infection. The therapy can be any of the therapeutic agents referred to herein. The therapy also can be non-drug treatments. In  
10       important embodiments, the therapy is one which increases levels of gelsolin. In a particularly important embodiment, the therapy is a therapy with gelsolin. Preferred subjects are human subjects. The subject most likely to benefit from this invention is a human subject on-therapy and who has a gelsolin level below about 2.4  $\mu\text{M/L}$  of plasma.

          In some embodiments, the subject already has or had an infection. A subject who  
15       has or has had a primary (first) bacterial, viral, fungal, parasitic, or protozoal infection may be at an elevated risk of a secondary (second) infection. In some embodiments, the subject has not had a primary infection, but is at an elevated risk of having an infection because the subject has one or more risk factors to have an infection. Risk factors for a primary infection include: immunosuppression, immunocompromise, age, trauma, burns  
20       (e.g., thermal burns), surgery, foreign bodies, cancer, newborns especially newborns born prematurely. The degree of risk of an infection depends on the multitude and the severity or the magnitude of the risk factors that the subject has. Risk charts and prediction algorithms are available for assessing the risk of an infection in a subject based on the presence and severity of risk factors.

25           Other methods of assessing the risk of an infection in a subject are known by those of ordinary skill in the art

          In still other embodiments, the subject has had a primary infection and has one or more other risk factors.

          The preferred treatment of the instant invention is gelsolin. Gelsolin may be  
30       administered alone, in a pharmaceutical composition or combined with other therapeutic regimens. Gelsolin and other therapeutic agent(s) may be administered simultaneously or sequentially. When the other therapeutic agents are administered simultaneously they

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can be administered in the same or separate formulations, but are administered at the same time. The other therapeutic agents may be administered sequentially with one another and with gelsolin when the administration of the other therapeutic agents and the gelsolin is temporally separated. The separation in time between the administration of these compounds may be a matter of minutes or it may be longer. Other therapeutic agents include but are not limited to anti-infective agent(s). Examples of anti-infective agent(s) include: anti-bacterial agent(s), anti-viral agent(s), anti-fungal agent(s) or anti-protozoal agent(s).

Phrases such as "anti-infective agent", "anti-bacterial agent", "anti-viral agent", "anti-fungal agent", "anti-parasitic agent" and "parasiticide" have well-established meanings to those of ordinary skill in the art and are defined in standard medical texts. Briefly, anti-bacterial agents kill or inhibit the growth or function of bacteria. Anti-bacterial agents include antibiotics as well as other synthetic or natural compounds having similar functions. Antibiotics, typically, are low molecular weight molecules which are produced as secondary metabolites by cells, such as microorganisms. In general, antibiotics interfere with one or more bacterial functions or structures which are specific for the microorganism and which are not present in host cells.

A large class of anti-bacterial agents is antibiotics. Antibiotics that are effective for killing or inhibiting a wide range of bacteria are referred to as broad spectrum antibiotics. Other types of antibiotics are predominantly effective against the bacteria of the class gram-positive or gram-negative. These types of antibiotics are referred to as narrow spectrum antibiotics. Other antibiotics which are effective against a single organism or disease and not against other types of bacteria, are referred to as limited spectrum antibiotics. Anti-bacterial agents are sometimes classified based on their primary mode of action. In general, anti-bacterial agents are cell wall synthesis inhibitors, cell membrane inhibitors, protein synthesis inhibitors, nucleic acid synthesis or functional inhibitors, and competitive inhibitors.

Anti-bacterial agents include but are not limited to aminoglycosides,  $\beta$ -lactam agents, cephalosporins, macrolides, penicillins, quinolones, sulfonamides, and tetracyclines. Examples of anti-bacterial agents include but are not limited to: Acedapsone, Acetosulfone Sodium, Alamecin, Alexidine, Amdinocillin Clavulanate Potassium, Amdinocillin, Amdinocillin Pivoxil, Amicycline, Amifloxacin, Amifloxacin

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- Mesylate, Amikacin, Amikacin Sulfate, Aminosalicyclic acid, Aminosalicylate sodium, Amoxicillin, Amphomycin, Ampicillin, Ampicillin Sodium, Apalcillin Sodium, Apramycin, Aspartocin, Astromicin Sulfate, Avilamycin, Avoparcin, Azithromycin, Azlocillin, Azlocillin Sodium, Bacampicillin Hydrochloride, Bacitracin, Bacitracin
- 5 Methylene Disalicylate, Bacitracin Zinc, Bambermycins, Benzoylpas Calcium, Berythromycin, Betamicin Sulfate, Biapenem, Biniramycin, Biphenamine Hydrochloride, Bispyrithione Magsulfex, Butikacin, Butirosin Sulfate, Capreomycin Sulfate, Carbadox, Carbenicillin Disodium, Carbenicillin Indanyl Sodium, Carbenicillin Phenyl Sodium, Carbenicillin Potassium, Carumonam Sodium, Cefaclor, Cefadroxil,
- 10 Cefamandole, Cefamandole Nafate, Cefamandole Sodium, Cefaparole, Cefatrizine, Cefazaflur Sodium, Cefazolin, Cefazolin Sodium, Cefbuperazone, Cefdinir, Cefditoren Pivoxil, Cefepime, Cefepime Hydrochloride, Cefetecol, Cefixime, Cefmenoxime Hydrochloride, Cefmetazole, Cefmetazole Sodium, Cefonicid Monosodium, Cefonicid Sodium, Cefoperazone Sodium, Ceforanide, Cefotaxime, Cefotaxime Sodium, Cefotetan,
- 15 Cefotetan Disodium, Cefotiam Hydrochloride, Cefoxitin, Cefoxitin Sodium, Cefpimizole, Cefpimizole Sodium, Cefpiramide, Cefpiramide Sodium, Cefpirome Sulfate, Cefpodoxime Proxetil, Cefprozil, Cefroxadine, Cefsulodin Sodium, Ceftazidime, Ceftazidime Sodium, Ceftibuten, Ceftizoxime Sodium, Ceftriaxone Sodium, Cefuroxime, Cefuroxime Axetil, Cefuroxime Pivoxetil, Cefuroxime Sodium,
- 20 Cephacetrile Sodium, Cephalixin, Cephalixin Hydrochloride, Cephaloglycin, Cephaloridine, Cephalothin Sodium, Cephapirin Sodium, Cephradine, Cetocycline Hydrochloride, Cetophenicol, Chloramphenicol, Chloramphenicol Palmitate, Chloramphenicol Pantothenate Complex, Chloramphenicol Sodium Succinate, Chlorhexidine Phosphanilate, Chloroxylenol, Chlortetracycline Bisulfate,
- 25 Chlortetracycline Hydrochloride, Cilastatin, Cinoxacin, Ciprofloxacin, Ciprofloxacin Hydrochloride, Cirolemycin, Clarithromycin, Clavulanate Potassium, Clinafloxacin Hydrochloride, Clindamycin, Clindamycin Dextrose, Clindamycin Hydrochloride, Clindamycin Palmitate Hydrochloride, Clindamycin Phosphate, Clofazimine, Cloxacillin Benzathine, Cloxacillin Sodium, Cloxyquin, Colistimethate, Colistimethate Sodium,
- 30 Colistin Sulfate, Coumermycin, Coumermycin Sodium, Cyclacillin, Cycloserine, Dalfopristin, Dapsone, Daptomycin, Demeclocycline, Demeclocycline Hydrochloride, Demecycline, Denofungin, Diaveridine, Dicloxacillin, Dicloxacillin Sodium,

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- Dihydrostreptomycin Sulfate, Dipyrithione, Dirithromycin, Doxycycline, Doxycycline Calcium, Doxycycline Fosfatex, Doxycycline Hyclate, Doxycycline Monohydrate, Droxacin Sodium, Enoxacin, Epicillin, Epitetracycline Hydrochloride, Ertapenem, Erythromycin, Erythromycin Acistrate, Erythromycin Estolate, Erythromycin
- 5 Ethylsuccinate, Erythromycin Gluceptate, Erythromycin Lactobionate, Erythromycin Propionate, Erythromycin Stearate, Ethambutol Hydrochloride, Ethionamide, Fleroxacin, Floxacillin, Fludalanine, Flumequine, Fosfomycin, Fosfomycin Tromethamine, Fumoxicillin, Furazolium Chloride, Furazolium Tartrate, Fusidate Sodium, Fusidic Acid, Gatifloxacin, Genifloxacin, Gentamicin Sulfate, Gloximonam, Gramicidin, Haloprogin,
- 10 Hetacillin, Hetacillin Potassium, Hexedine, Ibafoxacin, Imipenem, Isoconazole, Isepamicin, Isoniazid, Josamycin, Kanamycin Sulfate, Kitasamycin, Levofloxacin, Levofuraltadone, Levopropylcillin Potassium, Lexithromycin, Lincomycin, Lincomycin Hydrochloride, Linezolid, Lomefloxacin, Lomefloxacin Hydrochloride, Lomefloxacin Mesylate, Loracarbef, Mafenide, Meclocycline, Meclocycline Sulfosalicylate,
- 15 Megalomicin Potassium Phosphate, Mequidox, Meropenem, Methacycline, Methacycline Hydrochloride, Methenamine, Methenamine Hippurate, Methenamine Mandelate, Methicillin Sodium, Metioprime, Metronidazole Hydrochloride, Metronidazole Phosphate, Mezlocillin, Mezlocillin Sodium, Minocycline, Minocycline Hydrochloride, Mirincamycin Hydrochloride, Monensin, Monensin Sodium,
- 20 Moxifloxacin Hydrochloride, Nafcillin Sodium, Nalidixate Sodium, Nalidixic Acid, Natamycin, Nebramycin, Neomycin Palmitate, Neomycin Sulfate, Neomycin Undecylenate, Netilmicin Sulfate, Neutramycin, Nifuradene, Nifuraldezone, Nifuratel, Nifuratrone, Nifurdazil, Nifurimide, Nifurpirinol, Nifurquinazol, Nifurthiazole, Nitrocyline, Nitrofurantoin, Nitromide, Norfloxacin, Novobiocin Sodium, Ofloxacin,
- 25 Ormetoprim, Oxacillin Sodium, Oximonam, Oximonam Sodium, Oxolinic Acid, Oxytetracycline, Oxytetracycline Calcium, Oxytetracycline Hydrochloride, Paldimycin, Parachlorophenol, Paulomycin, Pefloxacin, Pefloxacin Mesylate, Penamcillin, Penicillin G Benzathine, Penicillin G Potassium, Penicillin G Procaine, Penicillin G Sodium, Penicillin V, Penicillin V Benzathine, Penicillin V Hydrabamine, Penicillin V
- 30 Potassium, Pentizidone Sodium, Phenyl Aminosalicylate, Piperacillin, Piperacillin Sodium, Pirbenicillin Sodium, Piridicillin Sodium, Pirlimycin Hydrochloride, Pivampicillin Hydrochloride, Pivampicillin Pamoate, Pivampicillin Probenate,

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- Polymyxin B Sulfate, Porfiromycin, Propikacin, Pyrazinamide, Pyriithione Zinc, Quindecamine Acetate, Quinupristin, Racephenicol, Ramoplanin, Ranimycin, Relomycin, Repromicin, Rifabutin, Rifametane, Rifamexil, Rifamide, Rifampin, Rifapentine, Rifaximin, Rolitetracycline, Rolitetracycline Nitrate, Rosaramicin, Rosaramicin Butyrate, Rosaramicin Propionate, Rosaramicin Sodium Phosphate, Rosaramicin Stearate, Rosoxacin, Roxarsone, Roxithromycin, Sancycline, Sanfetrinem Sodium, Sarmoxicillin, Sarpicillin, Scopafungin, Sisomicin, Sisomicin Sulfate, Sparfloxacin, Spectinomycin Hydrochloride, Spiramycin, Stallimycin Hydrochloride, Steffimycin, Sterile Ticarcillin Disodium, Streptomycin Sulfate, Streptonicozid, Sulbactam Sodium, Sulfabenz, Sulfabenzamide, Sulfacetamide, Sulfacetamide Sodium, Sulfacytine, Sulfadiazine, Sulfadiazine Sodium, Sulfadoxine, Sulfalene, Sulfamerazine, Sulfameter, Sulfamethazine, Sulfamethizole, Sulfamethoxazole, Sulfamonomethoxine, Sulfamoxole, Sulfanilate Zinc, Sulfanitran, Sulfasalazine, Sulfasomizole, Sulfathiazole, Sulfazamet, Sulfisoxazole, Sulfisoxazole Acetyl, Sulfisoxazole Diolamine, Sulfomyxin, Sulopenem, Sultamicillin, Suncillin Sodium, Talampicillin Hydrochloride, Tazobactam, Teicoplanin, Temafloxacin Hydrochloride, Temocillin, Tetracycline, Tetracycline Hydrochloride, Tetracycline Phosphate Complex, Tetroxoprim, Thiamphenicol, Thiphencillin Potassium, Ticarcillin Cresyl Sodium, Ticarcillin Disodium, Ticarcillin Monosodium, Ticlatone, Tiodonium Chloride, Tobramycin, Tobramycin Sulfate, Tosufloxacin, Trimethoprim, Trimethoprim Sulfate, Trisulfapyrimidines, Troleandomycin, Trospetomycin Sulfate, Trovafloxacin, Tyrothricin, Vancomycin, Vancomycin Hydrochloride, Virginiamycin, Zorbamycin.

- Anti-viral agents can be isolated from natural sources or synthesized and are useful for killing or inhibiting the growth or function of viruses. Anti-viral agents are compounds which prevent infection of cells by viruses or replication of the virus within the cell. There are several stages within the process of viral infection which can be blocked or inhibited by anti-viral agents. These stages include, attachment of the virus to the host cell (immunoglobulin or binding peptides), uncoating of the virus (e.g. amantadine), synthesis or translation of viral mRNA (e.g. interferon), replication of viral RNA or DNA (e.g. nucleotide analogues), maturation of new virus proteins (e.g. protease inhibitors), and budding and release of the virus.

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Anti-viral agents useful in the invention include but are not limited to: immunoglobulins, amantadine, interferons, nucleotide analogues, and protease inhibitors.

Specific examples of anti-virals include but are not limited to Acemannan; Acyclovir;

Acyclovir Sodium; Adefovir; Alovudine; Alvircept Sudotox; Amantadine

5 Hydrochloride; Aranotin; Arildone; Atevirdine Mesylate; Avridine; Cidofovir;

Cipamfylline; Cytarabine Hydrochloride; Delavirdine Mesylate; Desciclovir;

Didanosine; Disoxaril; Edoxudine; Envirodene; Enviroxime; Famciclovir; Famotidine

Hydrochloride; Fiacitabine; Fialuridine; Fosarilate; Foscarnet Sodium; Fosfonet Sodium;

Ganciclovir; Ganciclovir Sodium; Idoxuridine; Kethoxal; Lamivudine; Lobucavir;

10 Memotidine Hydrochloride; Methisazone; Nevirapine; Penciclovir; Pirodavid; Ribavirin;

Rimantadine Hydrochloride; Saquinavir Mesylate; Somantadine Hydrochloride;

Sorivudine; Statolon; Stavudine; Tilorone Hydrochloride; Trifluridine; Valacyclovir

Hydrochloride; Vidarabine; Vidarabine Phosphate; Vidarabine Sodium Phosphate;

Viroxime; Zalcitabine; Zidovudine; and Zinviroxime.

15 Nucleotide analogues are synthetic compounds which are similar to nucleotides, but which have an incomplete or abnormal deoxyribose or ribose group. Once the nucleotide analogues are in the cell, they are phosphorylated, producing the triphosphate formed which competes with normal nucleotides for incorporation into the viral DNA or RNA. Once the triphosphate form of the nucleotide analogue is incorporated into the

20 growing nucleic acid chain, it causes irreversible association with the viral polymerase and thus chain termination. Nucleotide analogues include, but are not limited to, acyclovir (used for the treatment of herpes simplex virus and varicella-zoster virus), gancyclovir (useful for the treatment of cytomegalovirus), idoxuridine, ribavirin (useful for the treatment of respiratory syncytial virus), dideoxyinosine, dideoxycytidine,

25 zidovudine (azidothymidine), imiquimod, and resiquimod.

The interferons are cytokines which are secreted by virus-infected cells as well as immune cells. The interferons function by binding to specific receptors on cells adjacent to the infected cells, causing the change in the cell which protects it from infection by the virus.  $\alpha$  and  $\beta$ -interferon also induce the expression of Class I and Class II MHC

30 molecules on the surface of infected cells, resulting in increased antigen presentation for host immune cell recognition.  $\alpha$  and  $\beta$ -interferons are available as recombinant forms and have been used for the treatment of chronic hepatitis B and C infection. At the

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dosages which are effective for anti-viral therapy, interferons have severe side effects such as fever, malaise and weight loss.

Anti-fungal agents are used to treat superficial fungal infections as well as opportunistic and primary systemic fungal infections. Anti-fungal agents are useful for the treatment and prevention of infective fungi. Anti-fungal agents are sometimes  
5 classified by their mechanism of action. Some anti-fungal agents function, for example, as cell wall inhibitors by inhibiting glucose synthase. These include, but are not limited to, basiungin/ECB. Other anti-fungal agents function by destabilizing membrane integrity. These include, but are not limited to, imidazoles, such as clotrimazole, sertaconazole, fluconazole, itraconazole, ketoconazole, miconazole, and voriconazole, as  
10 well as FK 463, amphotericin B, BAY 38-9502, MK 991, pradimicin, UK 292, butenafine, and terbinafine. Other anti-fungal agents function by breaking down chitin (e.g. chitinase) or immunosuppression (501 cream).

Anti-parasitic agents kill or inhibit parasites. Examples of anti-parasitic agents, also referred to as parasiticides, useful for human administration include but are not  
15 limited to albendazole, amphotericin B, benznidazole, bithionol, chloroquine HCl, chloroquine phosphate, clindamycin, dehydroemetine, diethylcarbamazine, diloxanide furoate, eflornithine, furazolidone, glucocorticoids, halofantrine, iodoquinol, ivermectin, mebendazole, mefloquine, meglumine antimoniate, melarsoprol, metrifonate, metronidazole, niclosamide, nifurtimox, oxamniquine, paromomycin, pentamidine  
20 isethionate, piperazine, praziquantel, primaquine phosphate, proguanil, pyrantel pamoate, pyrimethamine-sulfonamides, pyrimethamine-sulfadoxine, quinacrine HCl, quinine sulfate, quinidine gluconate, spiramycin, stibogluconate sodium (sodium antimony gluconate), suramin, tetracycline, doxycycline, thiabendazole, tinidazole, trimethoprim-sulfamethoxazole, and tryparsamide some of which are used alone or in combination  
25 with others.

In practicing certain methods of the present invention, it is required to obtain a level of gelsolin in a subject. This level then is compared to a predetermined value, wherein the level of gelsolin in comparison to the predetermined value is indicative of  
30 the likelihood that the subject will benefit from continued therapy. The subject then can be characterized in terms of the net benefit likely to be obtained from a change in therapy.

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The level of the gelsolin for the subject can be obtained by any art recognized method. Typically, the level is determined by measuring the level of the marker in a body fluid, for example, blood, lymph, saliva, urine and the like. The level can be determined by ELISA, or immunoassays or other conventional techniques for  
5 determining the presence of the marker. Conventional methods include sending a sample(s) of a subject's body fluid to a commercial laboratory for measurement. Methods for measuring gelsolin are described in the Example.

The invention also involves comparing the level of gelsolin for the subject with a predetermined value. The predetermined value can take a variety of forms. It can be  
10 single cut-off value, such as a median or mean. It can be established based upon comparative groups, such as, for example, where the risk in one defined group is double the risk in another defined group. It can be a range, for example, where the tested population is divided equally (or unequally) into groups, such as a low-risk group, a medium-risk group and a high-risk group, or into quartiles, the lowest quartile being  
15 subjects with the highest risk and the highest quartile being subjects with the lowest risk, or into tertiles the lowest tertile being subjects with the highest risk and the highest tertile being subjects with the lowest risk.

The predetermined value can depend upon the particular population of subjects selected. For example, an apparently healthy population will have a different 'normal'  
20 range of gelsolin than will a population the subjects of which have had a prior infection or other condition. Accordingly, the predetermined values selected may take into account the category in which a subject falls. Appropriate ranges and categories can be selected with no more than routine experimentation by those of ordinary skill in the art.

The preferred body fluid is blood. One preferred predetermined value of gelsolin  
25 is about 2.4  $\mu\text{M/L}$  of plasma.

An important predetermined value of gelsolin is a value that is the average for a healthy subject population (i.e., subjects who have no signs and symptoms of disease). The predetermined value will depend, of course, upon the characteristics of the subject population in which the subject lies. In characterizing risk, numerous predetermined  
30 values can be established.



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Presently, there are commercial sources which produce reagents for assays for gelsolin. These include, for example, Cytoskeleton (Denver, CO), Sigma (St. Louis, MO) and Calbiochem (San Diego, CA)

5 The invention further comprises measuring the level of gelsolin together with a level of another marker of infection such as, for example, a level white blood cells (WBCs) for characterizing a subject's risk of developing an infection. A level of gelsolin in the subject is obtained. The level of gelsolin is compared to a predetermined value to establish a first risk value. A level of WBCs in the subject is also obtained. The level of the WBCs in the subject is compared to a second predetermined value to  
10 establish a second risk value. The subject's risk profile of developing an infection then is characterized based upon the combination of the first risk value and the second risk value, wherein the combination of the first risk value and second risk value establishes a third risk value different from the first and second risk values. In some embodiments, the third risk value is greater than either of the first and second risk values. The  
15 preferred subjects for testing and predetermined values are as described above. The infection may be any infection such as described above.

The invention provides methods for determining whether a subject will benefit from continued therapy or would benefit from a change in therapy. The benefit is typically a reduction in the rate of occurrence of an infection or a faster recovery from an  
20 infection. Determining whether a subject will benefit from continued therapy or would benefit from a change in therapy is clinically useful. One example of clinical usefulness of the methods of this invention includes identifying subjects who are less likely or more likely to respond to a therapy. The methods of the invention are also useful in predicting or determining that a subject would benefit from continued therapy or would benefit  
25 from a change in therapy. Another example of clinical usefulness, in the case of human subjects for example, includes aiding clinical investigators in the selection for clinical trials of subjects with a high likelihood of obtaining a net benefit. It is expected that clinical investigators now will use the present invention for determining entry criteria for clinical trials.

30 A subject who would benefit from continued therapy is a subject whose on-therapy level of gelsolin reaches a certain predetermined value or whose level of gelsolin is increasing. Predetermined values of gelsolin are described above. A subject who

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would benefit from a change in therapy is a subject whose on-therapy level of the gelsolin did not reach a certain predetermined value or whose on-therapy level of gelsolin is not increasing.

As used herein, a "change in therapy" refers to an increase or decrease in the dose  
5 of the existing therapy, a switch from one therapy to another therapy, an addition of another therapy to the existing therapy, or a combination thereof. A switch from one therapy to another may involve a switch to a therapy with a high risk profile but where the likelihood of expected benefit is increased. In some embodiments, preferred therapies are therapies that increase the levels of gelsolin. A subject who would benefit  
10 from a change in therapy by increasing the dose of the existing therapy is a subject who, for example, was on the therapy but was not receiving the maximum tolerated dose or the maximum allowed dose of the therapy and whose level of gelsolin did not reach a certain predetermined value. In such instances the dose of the existing therapy is increased until the level of gelsolin reaches a certain predetermined value. In some  
15 instances, the dose of the existing therapy is increased from the existing dose to a higher dose that is not the maximum tolerated dose nor the maximum allowed dose of the therapy. In other instances, the dose is increased to the maximum tolerated or to the maximum allowed dose of the therapy. A subject who would benefit from a change in therapy by decreasing the dose of the existing therapy is, for example, a subject whose  
20 on-therapy level of gelsolin reaches or can reach a certain predetermined value with a lower dose of the therapy.

A subject who would benefit from a switch from one therapy to another therapy is, for example, a subject who was on the maximum tolerated dose or the maximum allowed dose of the therapy and whose level of gelsolin did not reach a certain  
25 predetermined value. Another example is a subject was not on the maximum tolerated or the maximum allowed dose of the therapy but was determined by a health care practitioner to more likely benefit from another therapy. Such determinations are based, for example, on the development in the subject of unwanted side effects on the initial therapy or a lack of response to the initial therapy.

30 A subject who would benefit from a change in therapy by the addition of another therapy to the existing therapy is, for example, a subject who was on a therapy but whose level of gelsolin did not reach a certain predetermined value. In such instances, another

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therapy is added to the existing therapy. The therapy that is added to the existing therapy can have a different mechanism of action in increasing the level of gelsolin than the existing therapy. In some instances, a combination of the aforementioned changes in therapy may be used.

5           The invention also provides methods for determining the efficacy of a therapy. The efficacy is typically the efficacy of the therapy in increasing the level of gelsolin. This is sometimes also referred to as a positive response or a favorable response. Efficacy can be determined by a gelsolin blood test(s) to determine whether gelsolin  
10           levels are increased as a result of therapy. In some embodiments efficacy determination is based on the efficacy of a therapy in increasing both gelsolin and normalizing WBCs counts.

          The gelsolin measurement is reported in  $\mu\text{M/L}$  (micromoles/Liter), mg/dl (milligrams/deciliter), or mg/L (milligrams/Liter).

          The invention also provides methods for deciding on the course of a therapy in a  
15           subject undergoing therapy to treat an infection. Such a course of therapy is decided on the basis of the level of gelsolin. Therapies for treating or reducing the risk of an infection are described above. In some embodiments, the subject already has had an infection or is at risk of having an infection. A subject who has had a primary (first)  
20           infection is at an elevated risk of a secondary (second) infection due to the primary infection. In some embodiments, the subject is at an elevated risk of an infection because the subject has one or more risk factors to have an infection. Examples of risk factors to have an infection are described above. In some embodiments, the subject who is at an elevated risk of an infection may be an apparently healthy subject. An apparently  
25           healthy subject is a subject who has no signs or symptoms of disease.

          These methods have important implications for patient treatment and also for the  
25           clinical development of new therapies. It is also expected that clinical investigators now will use the present methods for determining entry criteria for human subjects in clinical trials. Health care practitioners select therapeutic regimens for treatment based upon the expected net benefit to the subject. The net benefit is derived from the risk to benefit  
30           ratio. The present invention permits the determination of whether a subject will benefit from continued therapy or would benefit from a change in therapy, thereby aiding the physician in selecting a therapy.

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The amount of a treatment may be varied for example by increasing or decreasing the amount of gelsolin or pharmacological agent or a therapeutic composition, by changing the therapeutic composition administered, by changing the route of administration, by changing the dosage timing and so on. The effective amount will vary  
5 with the particular infection or condition being treated, the age and physical condition of the subject being treated, the severity of the infection or condition, the duration of the treatment, the nature of the concurrent therapy (if any), the specific route of administration, and like factors are within the knowledge and expertise of the health practitioner. For example, an effective amount can depend upon the degree to which an  
10 individual has been exposed to or affected by exposure to the infection.

An effective amount is a dosage of the therapeutic agent sufficient to provide a medically desirable result. An effective amount may also, for example, depend upon the degree to which an individual has abnormally decreased levels of gelsolin. It should be understood that the therapeutic agents of the invention are used to treat or prevent  
15 infections, that is, they may be used prophylactically in subjects at risk of developing an infection. Thus, an effective amount is that amount which can lower the risk of, slow or perhaps prevent altogether the development of an infection. It will be recognized when the therapeutic agent is used in acute circumstances, it is used to prevent one or more medically undesirable results that typically flow from such adverse events.

The factors involved in determining an effective amount are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation. It is generally preferred that a maximum dose of the pharmacological agents of the invention (alone or in combination with other therapeutic agents) be used, that is, the highest safe dose according to sound medical judgment. It will be understood  
20 by those of ordinary skill in the art however, that a patient may insist upon a lower dose or tolerable dose for medical reasons, psychological reasons or for virtually any other reasons.

The therapeutically effective amount of a pharmacological agent of the invention is that amount effective to treat the disorder, such as an infection. In the case of  
30 infections the desired response is inhibiting the progression of the infection. This may involve only slowing the progression of the infection temporarily, although more preferably, it involves halting the progression of the infection permanently. This can be

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monitored by routine diagnostic methods known to those of ordinary skill in the art .  
The desired response to treatment of the infection also can be delaying the onset or even preventing the onset of the infection.

The pharmacological agents used in the methods of the invention are preferably  
5 sterile and contain an effective amount of gelsolin for producing the desired response in  
a unit of weight or volume suitable for administration to a subject. The doses of  
pharmacological agents administered to a subject can be chosen in accordance with  
different parameters, in particular in accordance with the mode of administration used  
and the state of the subject. Other factors include the desired period of treatment. In the  
10 event that a response in a subject is insufficient at the initial doses applied, higher doses  
(or effectively higher doses by a different, more localized delivery route) may be  
employed to the extent that patient tolerance permits. The dosage of a pharmacological  
agent may be adjusted by the individual physician or veterinarian, particularly in the  
event of any complication. A therapeutically effective amount typically varies from 0.01  
15 mg/kg to about 1000 mg/kg, preferably from about 0.1 mg/kg to about 200 mg/kg, and  
most preferably from about 0.2 mg/kg to about 20 mg/kg, in one or more dose  
administrations daily, for one or more days.

Various modes of administration are known to those of ordinary skill in the art  
which effectively deliver the pharmacological agents of the invention to a desired tissue,  
20 cell, or bodily fluid. The administration methods are discussed elsewhere in the  
application. The invention is not limited by the particular modes of administration  
disclosed herein. Standard references in the art (e.g., *Remington's Pharmaceutical  
Sciences*, 20th Edition, Lippincott, Williams and Wilkins, Baltimore MD, 2001) provide  
modes of administration and formulations for delivery of various pharmaceutical  
25 preparations and formulations in pharmaceutical carriers. Other protocols which are  
useful for the administration of pharmacological agents of the invention will be known to  
one of ordinary skill in the art, in which the dose amount, schedule of administration,  
sites of administration, mode of administration and the like vary from those presented  
herein.

30 Administration of pharmacological agents of the invention to mammals other  
than humans, e.g. for testing purposes or veterinary therapeutic purposes, is carried out  
under substantially the same conditions as described above. It will be understood by one

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of ordinary skill in the art that this invention is applicable to both human and animal diseases. Thus, this invention is intended to be used in husbandry and veterinary medicine as well as in human therapeutics.

When administered, the pharmaceutical preparations of the invention are applied  
5 in pharmaceutically-acceptable amounts and in pharmaceutically-acceptable compositions. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredients. Such preparations may routinely contain salts, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. When used in  
10 medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope of the invention. Such pharmacologically and pharmaceutically-acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic,  
15 acetic, salicylic, citric, formic, malonic, succinic, and the like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

A pharmacological agent or composition may be combined, if desired, with a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" as  
20 used herein means one or more compatible solid or liquid fillers, diluents or encapsulating substances which are suitable for administration into a human. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions also are capable of being co-mingled with the  
25 pharmacological agents of the invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

The pharmaceutical compositions may contain suitable buffering agents, as described above, including: acetate, phosphate, citrate, glycine, borate, carbonate, bicarbonate, hydroxide (and other bases) and pharmaceutically acceptable salts of the  
30 foregoing compounds. The pharmaceutical compositions also may contain, optionally, suitable preservatives, such as: benzalkonium chloride; chlorobutanol; parabens and thimerosal.

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The pharmaceutical compositions may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active agent into association with a carrier, which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing the active compound into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

Compositions suitable for oral administration may be presented as discrete units, such as capsules, tablets, pills, lozenges, each containing a predetermined amount of the active compound (e.g., gelsolin). Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir, an emulsion, or a gel.

Pharmaceutical preparations for oral use can be obtained as solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Optionally the oral formulations may also be formulated in saline or buffers, i.e. EDTA for neutralizing internal acid conditions or may be administered without any carriers.

Also specifically contemplated are oral dosage forms of the above component or components. The component or components may be chemically modified so that oral delivery of the derivative is efficacious. Generally, the chemical modification contemplated is the attachment of at least one moiety to the component molecule itself, where said moiety permits (a) inhibition of proteolysis; and (b) uptake into the blood stream from the stomach or intestine. Also desired is the increase in overall stability of the component or components and increase in circulation time in the body. Examples of such moieties include: polyethylene glycol, copolymers of ethylene glycol and propylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone and polyproline.

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Abuchowski and Davis, 1981, "Soluble Polymer-Enzyme Adducts" In: *Enzymes as Drugs*, Hosenberg and Roberts, eds., Wiley-Interscience, New York, NY, pp. 367-383; Newmark, et al., 1982, *J. Appl. Biochem.* 4:185-189. Other polymers that could be used are poly-1,3-dioxolane and poly-1,3,6-tioxocane. Preferred for pharmaceutical usage, as indicated  
5 above, are polyethylene glycol moieties.

For the component (or derivative) the location of release may be the stomach, the small intestine (the duodenum, the jejunum, or the ileum), or the large intestine. One skilled in the art has available formulations which will not dissolve in the stomach, yet will release the material in the duodenum or elsewhere in the intestine. Preferably, the release  
10 will avoid the deleterious effects of the stomach environment, either by protection of gelsolin or by release of the biologically active material beyond the stomach environment, such as in the intestine.

To ensure full gastric resistance a coating impermeable to at least pH 5.0 is essential. Examples of the more common inert ingredients that are used as enteric coatings  
15 are cellulose acetate trimellitate (CAT), hydroxypropylmethylcellulose phthalate (HPMCP), HPMCP 50, HPMCP 55, polyvinyl acetate phthalate (PVAP), Eudragit L30D, Aquateric, cellulose acetate phthalate (CAP), Eudragit L, Eudragit S, and Shellac. These coatings may be used as mixed films.

A coating or mixture of coatings can also be used on tablets, which are not intended  
20 for protection against the stomach. This can include sugar coatings, or coatings which make the tablet easier to swallow. Capsules may consist of a hard shell (such as gelatin) for delivery of dry therapeutic i.e. powder; for liquid forms, a soft gelatin shell may be used. The shell material of cachets could be thick starch or other edible paper. For pills, lozenges, molded tablets or tablet triturates, moist massing techniques can be used.

25 The therapeutic can be included in the formulation as fine multi-particulates in the form of granules or pellets of particle size about 1 mm. The formulation of the material for capsule administration could also be as a powder, lightly compressed plugs or even as tablets. The therapeutic could be prepared by compression.

Colorants and flavoring agents may all be included. For example, gelsolin may be  
30 formulated (such as by liposome or microsphere encapsulation) and then further contained within an edible product, such as a refrigerated beverage containing colorants and flavoring agents.



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One may dilute or increase the volume of the therapeutic with an inert material. These diluents could include carbohydrates, especially mannitol,  $\alpha$ -lactose, anhydrous lactose, cellulose, sucrose, modified dextrans and starch. Certain inorganic salts may be also be used as fillers including calcium triphosphate, magnesium carbonate and sodium chloride. Some commercially available diluents are Fast-Flo, Emdex, STA-Rx 1500, Emcompress and Avicell.

Disintegrants may be included in the formulation of the therapeutic into a solid dosage form. Materials used as disintegrants include but are not limited to starch, including the commercial disintegrant based on starch, Explotab. Sodium starch glycolate, Amberlite, sodium carboxymethylcellulose, ultramyopectin, sodium alginate, gelatin, orange peel, acid carboxymethyl cellulose, natural sponge and bentonite may all be used. Another form of the disintegrants are the insoluble cationic exchange resins. Powdered gums may be used as disintegrants and as binders and these can include powdered gums such as agar, Karaya or tragacanth. Alginic acid and its sodium salt are also useful as disintegrants.

Binders may be used to hold the therapeutic agent together to form a hard tablet and include materials from natural products such as acacia, tragacanth, starch and gelatin. Others include methyl cellulose (MC), ethyl cellulose (EC) and carboxymethyl cellulose (CMC). Polyvinyl pyrrolidone (PVP) and hydroxypropylmethyl cellulose (HPMC) could both be used in alcoholic solutions to granulate the therapeutic.

An anti-frictional agent may be included in the formulation of the therapeutic to prevent sticking during the formulation process. Lubricants may be used as a layer between the therapeutic and the die wall, and these can include but are not limited to; stearic acid including its magnesium and calcium salts, polytetrafluoroethylene (PTFE), liquid paraffin, vegetable oils and waxes. Soluble lubricants may also be used such as sodium lauryl sulfate, magnesium lauryl sulfate, polyethylene glycol of various molecular weights, Carbowax 4000 and 6000.

Glidants that might improve the flow properties of the drug during formulation and to aid rearrangement during compression might be added. The glidants may include starch, talc, pyrogenic silica and hydrated silicoaluminate.

To aid dissolution of the therapeutic into the aqueous environment a surfactant might be added as a wetting agent. Surfactants may include anionic detergents such as sodium lauryl sulfate, dioctyl sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic

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detergents might be used and could include benzalkonium chloride or benzethonium chloride. The list of potential non-ionic detergents that could be included in the formulation as surfactants are lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, polysorbate 40, 60, 65 and 80, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. These surfactants could be present in the formulation of gelsolin either alone or as a mixture in different ratios.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

Microspheres formulated for oral administration may also be used. Such microspheres have been well defined in the art. All formulations for oral administration should be in dosages suitable for such administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention may be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of *e.g.* gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

Also contemplated herein is pulmonary delivery of gelsolin. Gelsolin is delivered to the lungs of a mammal while inhaling and traverses across the lung epithelial lining to the blood stream. Other reports of inhaled molecules include Adjei et al., 1990, *Pharmaceutical Research*, 7:565-569; Adjei et al., 1990, *International Journal of Pharmaceutics*, 63:135-144 (leuprolide acetate); Braquet et al., 1989, *Journal of Cardiovascular Pharmacology*, 13(suppl. 5):143-146 (endothelin-1); Hubbard et al., 1989, *Annals of Internal Medicine*,

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Vol. III, pp. 206-212 ( $\alpha$ 1- antitrypsin); Smith et al., 1989, J. Clin. Invest. 84:1145-1146 ( $\alpha$ -1-proteinase); Oswein et al., 1990, "Aerosolization of Proteins", Proceedings of Symposium on Respiratory Drug Delivery II, Keystone, Colorado, March, (recombinant human growth hormone); Debs et al., 1988, J. Immunol. 140:3482-3488 (interferon- $\gamma$  and tumor necrosis factor alpha) and Platz et al., U.S. Patent No. 5,284,656 (granulocyte colony stimulating factor). A method and composition for pulmonary delivery of drugs for systemic effect is described in U.S. Patent No. 5,451,569, issued September 19, 1995 to Wong et al.

Contemplated for use in the practice of this invention are a wide range of mechanical devices designed for pulmonary delivery of therapeutic products, including but not limited to nebulizers, metered dose inhalers, and powder inhalers, all of which are familiar to those skilled in the art.

Some specific examples of commercially available devices suitable for the practice of this invention are the Ultravent nebulizer, manufactured by Mallinckrodt, Inc., St. Louis, Missouri; the Acorn II nebulizer, manufactured by Marquest Medical Products, Englewood, Colorado; the Ventolin metered dose inhaler, manufactured by Glaxo Inc., Research Triangle Park, North Carolina; and the Spinhaler powder inhaler, manufactured by Fisons Corp., Bedford, Massachusetts.

All such devices require the use of formulations suitable for the dispensing of gelsolin. Typically, each formulation is specific to the type of device employed and may involve the use of an appropriate propellant material, in addition to the usual diluents, adjuvants and/or carriers useful in therapy. Also, the use of liposomes, microcapsules or microspheres, inclusion complexes, or other types of carriers is contemplated. Chemically modified gelsolin may also be prepared in different formulations depending on the type of chemical modification or the type of device employed.

Formulations suitable for use with a nebulizer, either jet or ultrasonic, will typically comprise gelsolin dissolved in water at a concentration of about 0.1 to 25 mg of biologically active gelsolin per mL of solution. The formulation may also include a buffer and a simple sugar (e.g., for gelsolin stabilization and regulation of osmotic pressure). The nebulizer formulation may also contain a surfactant, to reduce or prevent surface induced aggregation of the gelsolin caused by atomization of the solution in forming the aerosol.

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Formulations for use with a metered-dose inhaler device will generally comprise a finely divided powder containing the gelsolin suspended in a propellant with the aid of a surfactant. The propellant may be any conventional material employed for this purpose, such as a chlorofluorocarbon, a hydrochlorofluorocarbon, a hydrofluorocarbon, or a hydrocarbon, including trichlorofluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol, and 1,1,1,2-tetrafluoroethane, or combinations thereof. Suitable surfactants include sorbitan trioleate and soya lecithin. Oleic acid may also be useful as a surfactant.

Formulations for dispensing from a powder inhaler device will comprise a finely divided dry powder containing gelsolin and may also include a bulking agent, such as lactose, sorbitol, sucrose, or mannitol in amounts which facilitate dispersal of the powder from the device, *e.g.*, 50 to 90% by weight of the formulation. The gelsolin should most advantageously be prepared in particulate form with an average particle size of less than 10 mm (or microns), most preferably 0.5 to 5 mm, for most effective delivery to the distal lung.

Nasal (or intranasal) delivery of a pharmaceutical composition of the present invention is also contemplated. Nasal delivery allows the passage of a pharmaceutical composition of the present invention to the blood stream directly after administering the therapeutic product to the nose, without the necessity for deposition of the product in the lung. Formulations for nasal delivery include those with dextran or cyclodextran.

For nasal administration, a useful device is a small, hard bottle to which a metered dose sprayer is attached. In one embodiment, the metered dose is delivered by drawing the pharmaceutical composition of the present invention solution into a chamber of defined volume, which chamber has an aperture dimensioned to aerosolize and aerosol formulation by forming a spray when a liquid in the chamber is compressed. The chamber is compressed to administer the pharmaceutical composition of the present invention. In a specific embodiment, the chamber is a piston arrangement. Such devices are commercially available.

Alternatively, a plastic squeeze bottle with an aperture or opening dimensioned to aerosolize an aerosol formulation by forming a spray when squeezed is used. The opening is usually found in the top of the bottle, and the top is generally tapered to partially fit in the nasal passages for efficient administration of the aerosol formulation.

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Preferably, the nasal inhaler will provide a metered amount of the aerosol formulation, for administration of a measured dose of the drug.

The compounds, when it is desirable to deliver them systemically, may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Alternatively, the active compounds may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal or vaginal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols.

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Suitable liquid or solid pharmaceutical preparation forms are, for example, aqueous or saline solutions for inhalation, microencapsulated, encochleated, coated onto microscopic gold particles, contained in liposomes, nebulized, aerosols, pellets for implantation into the skin, or dried onto a sharp object to be scratched into the skin. The pharmaceutical compositions also include granules, powders, tablets, coated tablets, (micro)capsules, suppositories, syrups, emulsions, suspensions, creams, drops or preparations with protracted release of active compounds, in whose preparation excipients and additives and/or auxiliaries such as disintegrants, binders, coating agents, swelling agents, lubricants, flavorings, sweeteners or solubilizers are customarily used as described above. The pharmaceutical compositions are suitable for use in a variety of drug delivery systems. For a brief review of methods for drug delivery, see Langer, *Science* 249:1527-1533, 1990, which is incorporated herein by reference.

Gelsolin and optionally other therapeutics may be administered *per se* or in the form of a pharmaceutically acceptable salt.

The therapeutic agent(s), including specifically but not limited to gelsolin, may be provided in particles. Particles as used herein means nano or microparticles (or in some instances larger) which can consist in whole or in part of gelsolin or the other therapeutic agent(s) as described herein. The particles may contain the therapeutic agent(s) in a core surrounded by a coating, including, but not limited to, an enteric coating. The therapeutic agent(s) also may be dispersed throughout the particles. The therapeutic agent(s) also may be adsorbed into the particles. The particles may be of any order release kinetics, including zero order release, first order release, second order release, delayed release, sustained release, immediate release, and any combination thereof, etc. The particle may include, in addition to the therapeutic agent(s), any of those materials routinely used in the art of pharmacy and medicine, including, but not limited to, erodible, nonerodible, biodegradable, or nonbiodegradable material or combinations thereof. The particles may be microcapsules which contain the gelsolin in a solution or in a semi-solid state. The particles may be of virtually any shape.

Both non-biodegradable and biodegradable polymeric materials can be used in the manufacture of particles for delivering the therapeutic agent(s). Such polymers may be natural or synthetic polymers. The polymer is selected based on the period of time over which release is desired. Bioadhesive polymers of particular interest include

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bioerodible hydrogels described by H.S. Sawhney, C.P. Pathak and J.A. Hubell in *Macromolecules*, (1993) 26:581-587, the teachings of which are incorporated herein. These include polyhyaluronic acids, casein, gelatin, glutin, polyanhydrides, polyacrylic acid, alginate, chitosan, poly(methyl methacrylates), poly(ethyl methacrylates),  
5 poly(butylmethacrylate), poly(isobutyl methacrylate), poly(hexylmethacrylate), poly(isodecyl methacrylate), poly(lauryl methacrylate), poly(phenyl methacrylate), poly(methyl acrylate), poly(isopropyl acrylate), poly(isobutyl acrylate), and poly(octadecyl acrylate).

The therapeutic agent(s) may be contained in controlled release systems. The  
10 term "controlled release" is intended to refer to any drug-containing formulation in which the manner and profile of drug release from the formulation are controlled. This refers to immediate as well as non-immediate release formulations, with non-immediate release formulations including but not limited to sustained release and delayed release formulations. The term "sustained release" (also referred to as "extended release") is used  
15 in its conventional sense to refer to a drug formulation that provides for gradual release of a drug over an extended period of time, and that preferably, although not necessarily, results in substantially constant blood levels of a drug over an extended time period. The term "delayed release" is used in its conventional sense to refer to a drug formulation in which there is a time delay between administration of the formulation and the release of  
20 the drug therefrom. "Delayed release" may or may not involve gradual release of drug over an extended period of time, and thus may or may not be "sustained release."

Use of a long-term sustained release implant may be particularly suitable for treatment of chronic conditions. "Long-term" release, as used herein, means that the implant is constructed and arranged to deliver therapeutic levels of the active ingredient  
25 for at least 7 days, and preferably 30-60 days. Long-term sustained release implants are well-known to those of ordinary skill in the art and include some of the release systems described above.

The invention also contemplates the use of kits. In some aspects of the invention, the kit can include a pharmaceutical preparation vial, a pharmaceutical preparation  
30 diluent vial, and gelsolin. The vial containing the diluent for the pharmaceutical preparation is optional. The diluent vial contains a diluent such as physiological saline for diluting what could be a concentrated solution or lyophilized powder of gelsolin. The

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instructions can include instructions for mixing a particular amount of the diluent with a particular amount of the concentrated pharmaceutical preparation, whereby a final formulation for injection or infusion is prepared. The instructions may include instructions for treating a subject with an effective amount of gelsolin. It also will be understood that the containers containing the preparations, whether the container is a bottle, a vial with a septum, an ampoule with a septum, an infusion bag, and the like, can contain indicia such as conventional markings which change color when the preparation has been autoclaved or otherwise sterilized.

The present invention is further illustrated by the following Example, which in no way should be construed as further limiting. The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference.

#### EXAMPLE

Sepsis is associated with various biochemical abnormalities, including plasma gelsolin depletion. While the true function of plasma gelsolin is not known, clinical and animal studies have shown that depletion of plasma gelsolin by injury and inflammation associates with adverse outcomes. We examined plasma gelsolin in septic mice and found that plasma gelsolin depletion occurs after septic challenges and that significant depletion accompanies lethal sepsis. Repletion of plasma gelsolin leads to a more favorable cytokine profile and improves mortality. Plasma gelsolin has a physiologic role in systemic inflammation and gelsolin replacement may represent a new therapy for infections and sepsis.

Gelsolin, specifically cytoplasmic gelsolin (cGSN), first discovered as an intracellular actin-binding protein involved in cell motility (Yin, H. L. & Stossel, T. P. (1979) *Nature* 281, 583-6) is also an abundant secretory protein (Yin, H. L., Kwiatkowski, D. J., Mole, J. E. & Cole, F. S. (1984) *J Biol Chem* 259, 5271-6). The exported isoform of gelsolin, designated plasma gelsolin (pGSN), has 25 additional amino acids and originates from alternative splicing of a single gene (Kwiatkowski, D. J., Stossel, T. P., Orkin, S. H., Mole, J. E., Colten, H. R. & Yin, H. L. (1986) *Nature* 323, 455-8). Although postulated as an "actin-scavenger" (Lee, W. M. & Galbraith, R. M.



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(1992) *N Engl J Med* 326, 1335-41), pGSN's biologic function is a mystery. pGSN's prevalence in complex organisms, including drosophila (Stella, M. C., Schauerte, H., Straub, K. L. & Leptin, M. (1994) *J Cell Biol* 125, 607-16), is consistent with it having an important physiologic role. In humans, trauma, massive hemolysis, acute respiratory distress syndrome (ARDS), hematopoietic stem cell transplantation (HSCT), acute hepatic failure, myonecrosis, pancreatitis, and sepsis can lead to pGSN depletion (Dahl, B., Schioldt, F. V., Ott, P., Gvozdenovic, R., Yin, H. L. & Lee, W. M. (1999) *Shock* 12, 102-4; Suhler, E., Lin, W., Yin, H. L. & Lee, W. M. (1997) *Crit Care Med* 25, 594-8; DiNubile, M. J., Stossel, T. P., Ljunghusen, O. C., Ferrara, J. L. & Antin, J. H. (2002) *Blood* 100, 4367-71; and Lind, S. E., Smith, D. B., Janney, P. A. & Stossel, T. P. (1988) *Am Rev Respir Dis* 138, 429-34). Furthermore, in trauma patients and HSCT recipients, lower pGSN levels predict increased morbidity and mortality (DiNubile, M. J., Stossel, T. P., Ljunghusen, O. C., Ferrara, J. L. & Antin, J. H. (2002) *Blood* 100, 4367-71; Mounzer, K. C., Moncure, M., Smith, Y. R. & Dinubile, M. J. (1999) *Am J Respir Crit Care Med* 160, 1673-81).

In animals, burns and acute lung injury induced by oxidative stress, radiation and hyperoxia also cause pGSN depletion (Rothenbach, P. A., Dahl, B., Schwartz, J. J., O'Keefe, G. E., Yamamoto, M., Lee, W. M., Horton, J. W., Yin, H. L. & Turnage, R. H. (2004) *J Appl Physiol* 96, 25-31; Christofidou-Solomidou, M., Scherpereel, A., Solomides, C. C., Christie, J. D., Stossel, T. P., Goelz, S. & DiNubile, M. J. (2002) *J Invest Med* 50, 54-60; and Christofidou-Solomidou, M., Scherpereel, A., Solomides, C. C., Muzykantov, V. R., Machtay, M., Albelda, S. M. & DiNubile, M. J. (2002) *Lung* 180, 91-104). Administration of pGSN to some of these animals lessens the injuries (Rothenbach, P. A., Dahl, B., Schwartz, J. J., O'Keefe, G. E., Yamamoto, M., Lee, W. M., Horton, J. W., Yin, H. L. & Turnage, R. H. (2004) *J Appl Physiol* 96, 25-31; and Christofidou-Solomidou, M., Scherpereel, A., Solomides, C. C., Christie, J. D., Stossel, T. P., Goelz, S. & DiNubile, M. J. (2002) *J Invest Med* 50, 54-60).

We hypothesized that if systemic inflammation associated with sepsis depresses pGSN, restoration of pGSN could be beneficial. Here, we show that pGSN levels fall in mice subjected to endotoxemia or peritonitis, and repletion of pGSN leads to improved survival and cytokine profile shift in septic mice.

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## Materials and Methods

### *Animals*

Wild type C57BL/6 male mice were purchased from Charles River Laboratories (Wilmington, MA). Toll-like receptor 4 (TLR4) mutants C3H/HeJ male mice were  
5 purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were given free access to a standard feed and water and all procedures and studies described here have been approved by Harvard Medical Area Standing Committee on Animals according to standards as set forth in The Guide for the Care and Use of Laboratory Animals.

### *LPS Dose Response*

10 6-8 week old male C57BL/6 mice weighing 18-20g were injected intraperitoneally (i.p.) with LPS (*Pseudomonas aeruginosa* Serotype 10, from Sigma (St. Louis, MO) at doses of 0, 10, 20, and 40mg/kg in 100 µl of phosphate buffered saline (PBS), and 3-4 were used in each group. 24 hours after LPS administration, animals were anesthetized with 0.015-0.017mg/g Avertin i.p. (Fluka Chemie, Buchs,  
15 Switzerland). Blood was then collected by retro-orbital bleeding into 0.1 volume of Aster-Jandl anticoagulant solution (Gamulescu, M. A., Seifert, K., Tingart, M., Falet, H. & Hoffmeister, K. M. (2003) *Platelets* 14, 211-7) and centrifuged at 1000 x g for 10 minutes to generate plasma. Plasma was frozen in liquid nitrogen and stored at -80°C.

### *Murine Sepsis by CLP (Cecal Ligation and Puncture)*

20 8-10 week old male C57BL/6 mice were first anesthetized with 0.015-0.017mg/g i.p. Avertin. The cecum of each anesthetized animal was exposed thru a small incision in the lower anterior abdomen, and punctured by a 19-gauge needle. A small amount of intestinal content was extruded and the cecum was ligated without obstructing intestinal tract with 6-0 silk suture. After replacing the intestinal contents, the abdomen was  
25 closed with a 4-0 silk suture. For pGSN level study, the 5 animals received 1 ml of PBS subcutaneously immediately after surgery. Five animals that did not undergo CLP served as controls. Animals were allowed to recover with free access to food and water. 24 hours after CLP, animals were then anesthetized and plasma collected as described before. In addition, heart, lungs, liver, kidneys and skeletal muscles from hind leg were  
30 harvested and frozen at -80°C from each animal. Lungs were harvested after perfusion by first puncturing the right ventricle of the heart and injecting 1 ml of PBS into the left ventricle. For the mortality study, 20 animals were subjected to CLP and 10 animals

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received subcutaneous (s.c.) injections of 1 ml of 1) 150 mM NaCl (saline) or 2) 8mg/ml of recombinant human pGSN (Biogen, Boston, MA) with 0.4 mM Ca in saline immediately and 24 hours after CLP.

#### *LPS Mortality and Plasma Cytokines*

5 6-8 week old male C57BL/6 mice weighing 18-20g were injected i.p. with 25mg/kg LPS (*Escherichia coli* O55:B5, St. Louis, Sigma) and divided to receive 400 $\mu$ l dorsal subcutaneous injection of 1) pGSN: 20mg/ml of recombinant human pGSN with 1mM Ca in saline (8 animals), 2) BSA: 20mg/ml of bovine serum albumin (Serologicals, Norcross, GA) with 1mM Ca in saline (9 animals), or 3) saline: sterile saline alone (9  
10 animals), immediately, and at 24, 48, and 72 hours after LPS injections. The animals were monitored frequently and mortality was recorded for 7 days. Surviving mice were euthanized. In a separate experiment, mice received the same LPS challenge and were divided to receive pGSN or saline treatment as described and sacrificed for plasma and organ collections at 6 hours (5 mice per treatment group) and 24 hours (4 mice per  
15 treatment group) post LPS challenge. In addition, control mice without LPS challenge were given only s.c. saline (5 mice) or pGSN (3 mice) 24 hours prior to being sacrificed for blood and organ harvesting. Plasma and organs were collected from each mouse as described.

#### *pGSN in LPS-Resistant Mice*

20 6-8 week old male C3H/HeJ mice weighing 19-20 g were injected i.p. with 25mg/kg *E. coli* LPS (4 mice) and unchallenged mice served as controls (4 mice). 24 hours after LPS challenge, plasma samples were collected from anesthetized mice as described above. Gelsolin level was measured in each plasma sample.

#### *Mouse Cytokine Measurements*

25 Plasma GM-CSF, INF- $\gamma$ , IL-1 $\beta$ , IL-6, IL-10, and TNF- $\alpha$  cytokines were measured using ELISA assays (LINCO Research, St. Charles, MO). The lower range of the assay is  $\leq 3.2$ pg/ml for each cytokine, and levels  $\leq 3.2$  pg/ml were assigned a value of zero.

#### *Gelsolin and Albumin Measurements*

30 Plasma gelsolin was measured in duplicate samples by its ability to stimulate actin nucleation (Janmey, P. A., Chaponnier, C., Lind, S. E., Zaner, K. S., Stossel, T. P. & Yin, H. L. (1985) *Biochemistry* 24, 3714-23). Mouse plasma was

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diluted 1:5 fold in 0.1 M KCl, 0.2 mM MgCl<sub>2</sub>, 1 mM EGTA, 0.5 mM ATP, 0.5 mM  $\beta$ -mercaptoethanol, 10 mM Tris-HCl buffer, pH 7.4 (Buffer B). Of the diluted plasma sample, 5  $\mu$ l was added to 280  $\mu$ l Buffer B supplemented with 1.5 mM CaCl<sub>4</sub> and 0.4  $\mu$ M Phalloidin in 6 x 50 mm borosilicate culture tubes. The actin polymerization reaction was initiated by adding 15  $\mu$ l 20  $\mu$ M pyrene actin in 0.5 mM ATP, 5 mM  $\beta$ -mercaptoethanol, 0.2 mM CaCl<sub>2</sub>, 0.2 mM Tris-HCl buffer, pH 7.4 (Buffer A). Polymerization was monitored for 200 seconds in a spectrofluorimeter at excitation and emission wavelengths of 366 and 386 nm respectively. Gelsolin concentrations were estimated from a standard curve using recombinant human pGSN. Stock pyrene actin for these assays, prepared by the method of Kouyama and Mihashi (Kouyama, T. & Mihashi, K. (1981) *Eur J Biochem* 114, 33-8), was stored at -80°C in lots, thawed and diluted 10 x with Buffer A, centrifuged at 250,000 x g for 30 minutes after standing overnight.

Gelsolin quantification by the actin nucleation assay correlates well with levels obtained from Western blotting measurements (Mounzer, K. C., Moncure, M., Smith, Y. R. & Dinubile, M. J. (1999) *Am J Respir Crit Care Med* 160, 1673-81). The assay is a highly specific, as evidenced by virtually zero activity in plasma of LPS treated gelsolin-null mice (data not shown); however, the assay does not discriminate between cGSN and pGSN. It is also not species-specific and is thus able to approximate total gelsolin levels in mice treated with recombinant human pGSN. Lipids complexing to pGSN do not affect pGSN's actin nucleation activity (Janmey, P. A., Iida, K., Yin, H. L. & Stossel, T. P. (1987) *J Biol Chem* 262, 12228-36).

Albumin levels were measured colorimetrically using a commercial kit (Stanbio, Boerne, TX) according to the manufacture's instruction.

#### 25 *Protein Extraction from Organs*

Organs collected 6hr after being challenged with 25 mg/kg i.p. *E coli* LPS, or CLP were analyzed. Organs from unchallenged mice served as controls. Each organ was homogenized in RIPA buffer (Boston Bioproducts, Ashland, MA), supplemented with protein inhibitor cocktail (Calbiochem, La Jolla, CA) at 1:100 concentration and sodium orthovanadate and incubated on ice for 30 minutes before centrifugation at 2,000 x g for 30 minutes at 4°C. The supernatant was removed and centrifuged again at 10,000 x g for 30 minutes at 4°C. The supernatant was removed and kept at -80°C till analysis.

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### *Western Blot Analysis*

Protein concentration was determined for each sample using DC Protein Assay (Bio-Rad, Hercules, CA) following the manufacturer's instructions. 10 µg of each sample was heated at 85°C for 3 minutes in SDS-sample buffer (Boston Bioproducts) then analyzed by SDS-PAGE using 12% Tris-Glycine Gel (Invitrogen, Carlsbad, CA) and transferred to a PVDF membrane (Millipore, Bedford, MA). After blocking the membrane overnight in 5% non-fat dry milk in Tris-buffered saline (TBS) with 0.05% Tween 20, mouse pGSN anti-sera was added at 1:1000 and then probed with HRP-linked anti-rabbit IgG's (Cell Signaling, Beverly, MA) at 1:2000. Chemiluminescence was developed with LumiGLO (Cell Signaling, Beverly, MA) and photofilm was exposed and developed. The anti-mouse pGSN sera was produced by immunizing rabbits against a peptide derived from the plasma extension of mouse pGSN using a commercial service (Invitrogen, Carlsbad, CA). The specificity and sensitivity of the anti-sera has been tested using ELISA and Western Blotting shown to be specific against only mouse pGSN and not cytoplasmic gelsolin (cGSN) (Data not shown).

### *Gelsolin-LPS Binding*

All studies were done in duplicates. Each well of a Microlite 2, white 96-well flat-bottom plate (Dynex Technologies, Chantilly, VA) was coated with various amount of recombinant human pGSN or BSA and incubated at 4°C overnight. After 4 washes with PB buffer (145 mM NaCl, 5 mM KCl, 2 mM MgCl<sub>2</sub>, 3.5 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM glucose, 10 mM Hepes, 3mg/ml BSA, 1mM CaCl<sub>2</sub>, pH 7.4), 2 µg of Alexa488-labeled LPS (*Escherichia coli* serotype 055:B5, Molecular Probes, Eugene, OR) was added to each well with 100 µl PB buffer and incubated at room temperature for 1 hour. After 4 washes with PB buffer, fluorescence of each well was analyzed in a spectrofluorimeter at excitation and emission wavelengths of 488 and 520 nm respectively. Amount of Alexa488-LPS bound was estimated by extrapolating from a standard curve generated by seeding various amounts of Alexa488-LPS in PB buffer.

### *LPS Stimulation of Monocytic Cells*

Human monocytic cell line THP-1 was purchased from American Type Culture Collection, Manassas, VA. Cells were maintained in RPMI (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum and 2% penicillin-streptomycin (GIBCO) at 37°C. 50,000 cells were seeded into each well in a 24-well plate and stimulated with or

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without 100 ng *E. coli* LPS and treated with 200 µg/ml human recombinant pGSN or BSA. 2 hours after LPS, 200 µl of media was collected and cells were removed by centrifugation at 1000 x g for 10 minutes. TNF-α levels of cell-free media were determined by ELISA (R&D Systems, Minneapolis, MN).

#### 5        *Statistics.*

Values are presented as mean ± SD. A nonparametric test, the Spearman Rank Correlation was used to analyze correlations in the dose response study. Animal mortality is presented as Kaplan-Meier curves, and the log-rank test was used to analyze treatment impact on animal mortality. The Mann-Whiney U test was used to evaluate differences between cytokine and pGSN levels. A P value less than 0.05 was considered significant.

### Results

#### *pGSN Levels Decrease in Mice Subjected to LPS or CLP*

15        Figure 1A shows that injection of increasing non-lethal doses of *Pseudomonas* LPS led to a progressive decrease, maximal at a dose of 20mg/kg, of pGSN levels in mice (P<0.05). Plasma albumin levels did not alter with LPS treatment. Similarly, pGSN levels fell (P<0.001) while albumin levels increased (P=0.02) in mice after CLP. These data suggest that systemic inflammation due to sepsis has a specific affect on pGSN.

#### 20        *Repletion of pGSN Improved Survival in Septic Mice*

*E. coli* LPS at a dose of 25mg/kg i.p. induces >90% mortality in mice within 7 days of injection, and the surviving mice appear completely recovered and exhibit no signs of distress. As shown in Figure 2A, administration of exogenous pGSN at the time of LPS challenge significantly enhanced survival in endotoxemic mice compared to those treated with saline (P<0.001), or BSA (P<0.001). Figure 2B shows that mice received pGSN also had significantly better survival than those received saline after CLP (P=0.001).

#### *Administration of Exogenous pGSN Effectively Raised pGSN Levels in Endotoxemic Mice*

30        Figure 3 shows the level of pGSN in mice challenged with lethal dose of *E. coli* LPS. In contrast to non-lethal *Pseudomonas* LPS which only caused pGSN to drop by 14%, a lethal dose of *E. coli* LPS induced a 50% drop in pGSN level within 6 hours of

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LPS challenge. Subcutaneous injection of 8 mg recombinant pGSN successfully kept pGSN at or above the normal range in endotoxemic mice.

*Effects of pGSN Repletion on the Cytokine Profiles of Endotoxemic Mice*

We examined if administration of pGSN alters the cytokine profile of  
 5 endotoxemic mice. Figure 4A shows no differences in the plasma cytokine profile  
 between pGSN-treated and saline-treated endotoxemic mice at 6 hours after LPS ( $P>0.05$   
 for all cytokines shown). TNF- $\alpha$  levels were near baseline, consistent with published  
 reports showing TNF- $\alpha$  peaks and begins to fall within 2-3 hours of LPS challenge in  
 mice (Villa, P., Sartor, G., Angelini, M., Sironi, M., Conni, M., Gnocchi, P., Isetta, A.  
 10 M., Grau, G., Buurman, W., van Tits, L. J. & et al. (1995) *Clin Diagn Lab Immunol* 2,  
 549-53).

24 hours after LPS challenge, however, pGSN-treated mice had significantly  
 lower levels of several pro-inflammatory cytokines (GM-CSF, IFN- $\gamma$ , IL-1 $\beta$ ), although  
 IL-6 and TNF- $\alpha$  levels were not detectably different (Figure 4B). In addition, pGSN  
 15 treatment resulted in a significantly higher IL-10 level. pGSN does not appear to directly  
 stimulate IL-10 secretion as unchallenged mice with or without pGSN administration did  
 not have significantly different cytokine profiles; specifically, IL-10 was not increased in  
 pGSN-treated unchallenged mice (Data not shown).

*Tissue Distribution of pGSN*

20 A representative Western blot analysis of skeletal muscles, hearts, kidneys livers,  
 and lungs harvested from mice 6 hours after being treated with or without 25mg/kg *E.*  
*coli* LPS, using mouse pGSN anti-sera is shown in Figure 6. We found that lung has the  
 highest concentration of pGSN in both normal and endotoxemic states. Since lungs were  
 perfused with PBS to remove intravascular blood prior to harvesting, it is unlikely that  
 25 blood contamination explains our result. We found similar tissue distribution of pGSN  
 in mice subjected to CLP (Data not shown).

*pGSN Binds LPS But Does Not Inhibit LPS Activating Monocytes*

Since pGSN can bind bioactive lipids such as lysophosphatidic acid (Goetzl, E.  
 J., Lee, H., Azuma, T., Stossel, T. P., Turck, C. W. & Karliner, J. S. (2000) *J Biol Chem*  
 30 275, 14573-8), we explored the possibility that pGSN can also bind LPS. Figure 7  
 shows that pGSN specifically binds to fluorescent LPS while control protein, BSA,  
 exhibited little affinity for LPS. However, pGSN does not appear to interfere with LPS's

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ability to elicit TNF- $\alpha$  secretion from human monocytes since LPS-stimulated THP-1 cells treated with pGSN or BSA secreted similar amount of TNF- $\alpha$  into the culture media (Figure 8).

*pGSN Is Unaffected in TLR4-Mutant Mice Challenged with LPS*

5 To examine if LPS directly causes pGSN depletion, we studied pGSN levels in C3H/HeJ mice, a strain expressing mutated TLR4 that renders the mice resistant to LPS-induced inflammation (Beutler, B. & Poltorak, A. (2001) *Crit Care Med* 29, S2-6; discussion S6-7). Figure 5 shows that pGSN levels did not differ significantly between *E coli* LPS-challenged and unchallenged C3H/HeJ mice. Consistent with their known  
10 resistance to LPS, C3H/HeJ mice also appeared completely normal after LPS challenge.

*Discussion*

We have demonstrated here that significant pGSN depletion occurs after septic insult by endotoxin or peritonitis, and that exogenous pGSN dramatically improves the survival of septic mice. These data indicate that pGSN serves an important pro-survival  
15 function in sepsis.

The hypothesis of pGSN being an "actin-scavenger" stems from studies on cytoplasmic gelsolin (cGSN), and on one report demonstrating infusion of actin molecules into rats causing death. However, pGSN may not act as a scavenger for circulating actin in sepsis because early sepsis is not known to lead to actin release, and  
20 we have not been able to demonstrate gelsolin-actin complexes in the plasma of endotoxemic mice (unpublished data).

We explored the possibility that pGSN functions by directly interacting with LPS. Although pGSN can bind LPS, we found no evidence of pGSN interfering with LPS's ability to initiate inflammation in culture cells. Although pGSN was injected into  
25 mice immediately after LPS challenge, there was a delay in pGSN being delivered into the circulation due to subcutaneous route of administration. Therefore, it is unlikely that pGSN functions by direct LPS interference, and the early cytokine profiles of treated and untreated endotoxemic mice support this conclusion. It is possible that pGSN's ability to bind LPS alters a later event in LPS-induced inflammatory response. The similar early  
30 phase cytokine profiles of our control and pGSN-treated mice also suggest that pGSN is unlikely to function by interfering with early cytokine signaling, specifically TNF- $\alpha$ , since TNF- $\alpha$  inhibition in murine model of endotoxemia lowers plasma IL-1 $\beta$  and IL-6



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levels within 3-4 hours of LPS challenge (Fong, Y., Tracey, K. J., Moldawer, L. L., Hesse, D. G., Manogue, K. B., Kenney, J. S., Lee, A. T., Kuo, G. C., Allison, A. C., Lowry, S. F. & et al. (1989) *J Exp Med* 170, 1627-33). On the other hand, cytokine profiles of treated and untreated mice 24 hours after LPS shows that pGSN is able to decrease several pro-inflammatory cytokines by as much as 90% while up-regulating IL-10, a cytokine believed to have an anti-inflammatory role in sepsis and inflammation (Moore, K. W., de Waal Malefyt, R., Coffman, R. L. & O'Garra, A. (2001) *Annu Rev Immunol* 19, 683-765). Since lethal dose of LPS is associated with persistently elevated pro-inflammatory cytokines, such as IL-1 $\beta$  and IFN- $\gamma$  (Joshi, V. D., Kalvakolanu, D. V., Hebel, J. R., Hasday, J. D. & Cross, A. S. (2002) *Infect Immun* 70, 6896-903), we believe that the cytokine profile shift is consistent with pGSN promoting the resolution of inflammation.

Lung appears to have the highest concentration of pGSN compared to other organs by Western blot analysis, and may be the principle source of pGSN. This is in contrast to a previous study concluding that skeletal muscle as the main source of pGSN using Northern blot analysis (Kwiatkowski, D. J., Mehl, R., Izumo, S., Nadal-Ginard, B. & Yin, H. L. (1988) *J Biol Chem* 263, 8239-43). The contradiction may be due to the limited quantifying capability of Northern blot analysis or mRNA expression being not representative of protein production.

The lack of pGSN changes in LPS-resistant mice suggests that pGSN depletion is downstream to LPS activation of TLR4 and requires the initiation of the inflammatory cascade. However, the current study does not elucidate the mechanism of pGSN depletion in sepsis.

Since the discovery of gelsolin more than 20 years ago, majority of research have focused on cGSN and its interaction with actin (Kwiatkowski, D. J. (1999) *Curr Opin Cell Biol* 11, 103-8), and pGSN has received little attention. Our study shows that pGSN plays a critical role in systemic inflammation and administration of pGSN improves survival in murine sepsis and promotes a shift of cytokines toward the resolution of inflammation.

The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the invention. The present invention is not to be limited in scope by examples provided, since the examples are intended as a single illustration of

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one aspect of the invention and other functionally equivalent embodiments are within the scope of the invention. Various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims. The advantages  
5 and objects of the invention are not necessarily encompassed by each embodiment of the invention.

What is claimed is:

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CLAIMS

1. A method for treating a subject having or at risk of developing an infection comprising:
  - 5 administering gelsolin to a subject in need of such a treatment, in an effective amount to treat the infection, wherein the infection is caused by a gram-positive bacterium, an acid-fast bacillus, a spirochete, an actinomycete, a virus, a fungus, a parasite, Ureoplasma species, Mycoplasma species, Chlamydia species, and Pneumocystis species.
- 10 2. The method of claim 1, wherein the subject is otherwise free of indications calling for treatment with gelsolin.
3. The method of claim 1, wherein the gelsolin is plasma gelsolin (pGSN).
- 15 4. The method of claim 1, wherein the gram-positive bacterium is selected from the group consisting of: Pasteurella species, Staphylococcus species, Streptococcus species, Bacillus anthracis, Corynebacterium species, Diphtheroids species, Listeria species, Erysipelothrix species, and Clostridium species.
- 20 5. The method of claim 1, wherein the acid-fast bacillus is a Mycobacterium species.
6. The method of claim 1, wherein the spirochete is selected from the group consisting of Treponema species, Borrelia species, and Leptospira species.
- 25 7. The method of claim 1, wherein the virus is selected from the group consisting of: Retro viruses, human immunodeficiency viruses, Cytomegaloviruses, Picorna viruses, Polio viruses, hepatitis A virus, enteroviruses, Coxsackie viruses, rhinoviruses, echoviruses, Calciviruses, Toga viruses, equine encephalitis viruses, rubella viruses,  
30 Flaviviruses, dengue viruses, encephalitis viruses, yellow fever viruses, coronaviruses, Rhabdoviruses, vesicular stomatitis viruses, rabies viruses, Filoviruses, ebola virus, Paramyxo viruses, parainfluenza viruses, mumps virus, measles virus, respiratory

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syncytial virus, Orthomyxoviruses, influenza viruses, Hantaan viruses, bunga viruses, phleboviruses, Nairo viruses, Arena viruses, hemorrhagic fever viruses, reoviruses, orbiviruses, rotaviruses, Birnaviruses, Hepadnaviruses, Hepatitis B virus, parvoviruses, Papovaviruses, papilloma viruses, polyoma viruses, Adenoviruses, Herpes viruses, 5 varicella zoster virus, Pox viruses, variola viruses, vaccinia viruses, Iridoviruses, African swine fever viruses, delta hepatitis virus, non-A, non-B hepatitis virus, Hepatitis C, Norwalk viruses, astroviruses, and unclassified viruses.

8. The method of claim 1, wherein the fungus is selected from the group consisting 10 of: Cryptococcus species, Histoplasma species, Coccidioides species, Paracoccidioides species, Blastomyces species, Chlamydia species, Candida species, Sporothrix species, Aspergillus species, and fungi of mucormycosis.

9. The method of claim 1, wherein the parasite is selected from the group consisting 15 of: Plasmodium species, Toxoplasma species, Babesia species, Leishmania species, and Trypanosoma species

10. The method of claim 1, wherein the gelsolin is administered orally, sublingually, buccally, intranasally, intravenously, intramuscularly, intrathecally, intraperitoneally, 20 subcutaneously, intradermally, topically, rectally, vaginally, intrasynovially or intravitreously.

11. The method of claim 1, wherein the gelsolin is administered subsequent to 25 exposure of the subject to the infection.

12. The method of claim 1, wherein the gelsolin is administered prior to exposure of the subject to the infection.

13. A method for treating a subject having or at risk of developing a gram-negative 30 bacterial infection comprising:

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administering gelsolin to a subject in need of such a treatment, in an effective amount to treat the gram-negative bacterial infection, at a time subsequent to exposure to the infection.

- 5 14. The method of claim 13, wherein the gram-negative bacterial infection is caused by *Neisseria* species, *Branhamella* species, *Escherichia* species, *Enterobacter* species, *Proteus* species, *Pseudomonas* species, *Klebsiella* species, *Salmonella* species, *Shigella* species, *Serratia* species, *Acinetobacter* species, *Haemophilus* species, *Brucella* species, *Yersinia* species, *Francisella* species, *Pasturella* species, *Vibrio cholera* species,
- 10 *Flavobacterium* species, *Pseudomonas* species, *Campylobacter* species, *Bacteroides* species, *Fusobacterium* species, *Calymmatobacterium* species, *Streptobacillus* species, and *Legionella* species.
- 15 15. The method of claim 13, wherein the subject is otherwise free of indications calling for treatment with gelsolin.
16. The method of claim 13, wherein the gelsolin is plasma gelsolin (pGSN).
- 20 17. The method of claim 13, wherein the gelsolin is administered orally, sublingually, buccally, intranasally, intravenously, intramuscularly, intrathecally, intraperitoneally, subcutaneously, intradermally, topically, rectally, vaginally, intrasynovially, or intravitreally.
- 25 18. A method of treating or preventing the effects of lipopolysaccharide endotoxin (LPS) in a subject comprising:  
administering to the subject an effective amount of gelsolin at a time subsequent to LPS exposure to protect the subject against the effects of LPS.
- 30 19. The method of claim 18, wherein the subject is otherwise free of indications calling for treatment with gelsolin.

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20. A method of treating or preventing gram-negative bacterial septic shock in a subject exposed to lipopolysaccharide endotoxin (LPS) comprising:

administering gelsolin to the subject in an effective amount to treat the gram-negative bacterial septic shock in the subject.

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21. The method of claim 20, wherein the subject is otherwise free of indications calling for treatment with gelsolin.

22. The method of claim 20, wherein the gelsolin is plasma gelsolin (pGSN).

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23. A method for up-regulating interleukin (IL) expression in a subject comprising: administering gelsolin to the subject in an effective amount to up-regulate interleukin (IL) expression.

15

24. The method of claim 23, wherein the IL is IL-10.

25. The method of claim 23, wherein the subject is otherwise free of indications calling for treatment with gelsolin.

20

26. The method of claim 23, wherein the gelsolin is plasma gelsolin (pGSN).

27. A method for up-regulating interleukin (IL) expression *in vitro*, comprising: contacting a cell capable of expressing IL with a gelsolin in an effective amount to up-regulate interleukin (IL) expression in the cell.

25

28. The method of claim 27, wherein the IL is IL-10.

29. The method of claim 27, wherein the subject is otherwise free of indications calling for treatment with gelsolin.

30

30. The method of claim 27, wherein the gelsolin is plasma gelsolin (pGSN).

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31. A method for down-regulating pro-inflammatory cytokine expression in a subject, comprising:

administering gelsolin to a subject in an effective amount to down-regulate pro-inflammatory cytokine expression in the subject.

5

32. The method of claim 31, wherein the subject is otherwise free of indications calling for treatment with gelsolin.

33. The method of claim 31, wherein the gelsolin is plasma gelsolin (pGSN).

10

34. The method of claim 31, wherein the pro-inflammatory cytokine is IL-1 $\beta$ .

35. The method of claim 31, wherein the pro-inflammatory cytokine is IFN- $\alpha$ .

15

36. A method for down-regulating pro-inflammatory cytokine expression *in vitro*, comprising:

contacting a cell capable of expressing a pro-inflammatory cytokine with gelsolin in an effective amount to down-regulate pro-inflammatory cytokine expression in the cell.

20

37. The method of claim 36, wherein pro-inflammatory cytokine is IL-1 $\beta$ .

38. The method of claim 36, wherein the pro-inflammatory cytokine is IFN- $\alpha$ .

25

39. The method of claim 36, wherein the subject is otherwise free of indications calling for treatment with gelsolin.

40. The method of claim 36, wherein the gelsolin is plasma gelsolin (pGSN).

30

41. A method for treating a subject to reduce the risk of an infection comprising selecting a subject on the basis that the subject is known to have a below-normal level of gelsolin and

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administering gelsolin to the subject and/or an anti-infective agent in an amount effective to reduce the risk of the subject developing an infection.

42. The method of claim 41, wherein the subject is otherwise free of indications  
5 calling for treatment with gelsolin.

43. The method of claim 41, wherein the gelsolin is plasma gelsolin (pGSN).

44. The method of claim 41, wherein the subject is apparently healthy.

10

45. The method of claim 41, wherein the infection is caused by a bacterium, a virus, a fungus, or a parasite.

46. The method of claim 41, wherein the anti-infective agent is an anti-bacterial, an  
15 anti-viral, an anti-fungal, or an anti-parasitic agent.

47. A method for evaluating the efficacy of a therapy for treating or reducing the risk of an infection in a subject comprising:

(i) obtaining a level of gelsolin in a subject undergoing therapy with an agent to  
20 treat or reduce the risk of an infection,

(ii) comparing the level of gelsolin obtained in (i) to a predetermined value corresponding to a level of gelsolin in an apparently healthy control population, and

(iii) determining whether the level of gelsolin in (i) is above a predetermined level said determination being indicative of whether the therapy is efficacious.

25

48. The method of claim 47, wherein step (i) and step (ii) are repeated so as to monitor the subject's levels of gelsolin over time.

49. The method of claim 47, wherein the predetermined value is about 2.4  $\mu\text{M/L}$  of  
30 plasma or lower.



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50. The method of claim 47, wherein the infection is caused by a bacterium, a virus, a fungus, or a parasite.

51. The method of claim 47, wherein the anti-infective agent is an anti-bacterial, an anti-viral, an anti-fungal, or an anti-parasitic agent.

52. A method for deciding on the course of a therapy in a subject, comprising:  
(i) obtaining a level of gelsolin in a subject undergoing a therapy to treat or reduce the risk of an infection,  
(ii) comparing the level of gelsolin obtained in (i) to a predetermined value corresponding to a level of gelsolin in an apparently healthy control population,  
(iii) determining whether the level of gelsolin obtained in (i) is below the predetermined level, and  
(iv) deciding on the course of the therapy based on such determination.

53. The method of claim 52, wherein step (i) is repeated so as to monitor the subject's level of gelsolin over time.

54. The method of claim 52, wherein the predetermined value is about 2.4  $\mu\text{M/L}$  of plasma or lower.

55. A method for treating a subject with a decreased level of gelsolin comprising:  
(i) treating the subject with a first therapy for treating or reducing the risk of an infection,

(ii) obtaining a level of gelsolin in the subject,  
(iii) comparing the level of gelsolin obtained in (ii) to a predetermined value corresponding to a predetermined level of gelsolin in an apparently healthy control population, and, if the predetermined level of gelsolin is not reached,  
(iv) treating the subject with a second therapy for treating or reducing the risk of an infection, and repeating (ii) and (iii) until the predetermined level of gelsolin is reached.

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56. The method of claim 55, wherein the predetermined value is about 2.4  $\mu\text{M/L}$  of plasma or higher.

57. A method for characterizing a subject's risk profile of developing a future  
5 infection, comprising:  
    obtaining a level of gelsolin in the subject,  
    comparing the level of the gelsolin to a predetermined value, and  
    characterizing the subject's risk profile of developing an infection based upon the  
level of gelsolin in comparison to the predetermined value, wherein a level of gelsolin  
10 below the predetermined level is indicative that the subject is at an elevated risk of  
developing an infection, and wherein a level of gelsolin above the predetermined level is  
indicative that the subject is not at an elevated risk of developing an infection.

58. The method of claim 57, wherein the predetermined value is about 2.4  $\mu\text{M/L}$  of  
15 plasma or lower.

59. The method of claim 57, wherein the subject is an apparently healthy subject.

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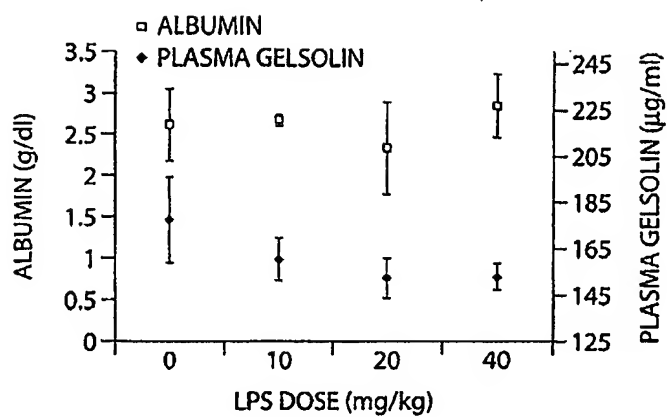


Fig. 1A

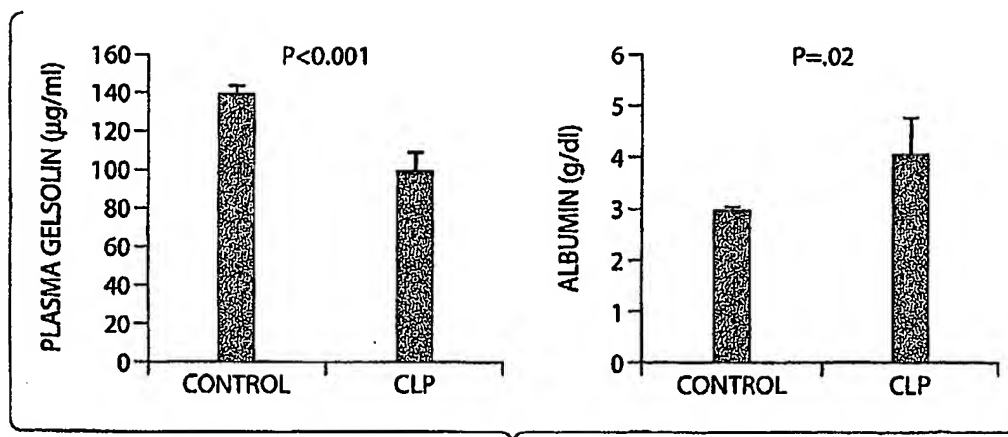


Fig. 1B

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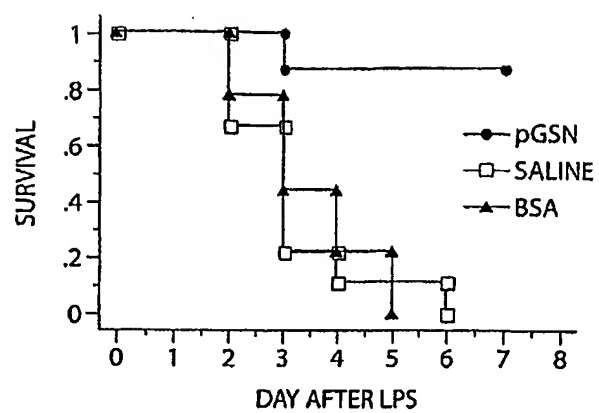


Fig. 2A

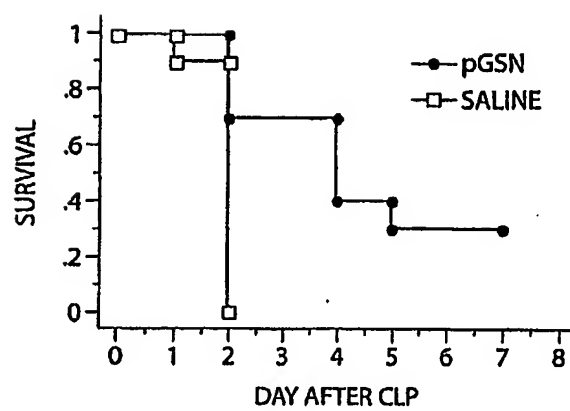


Fig. 2B

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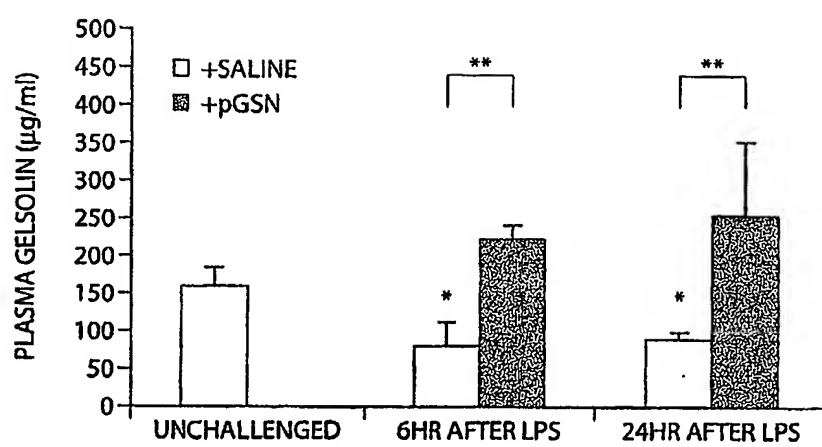


Fig. 3

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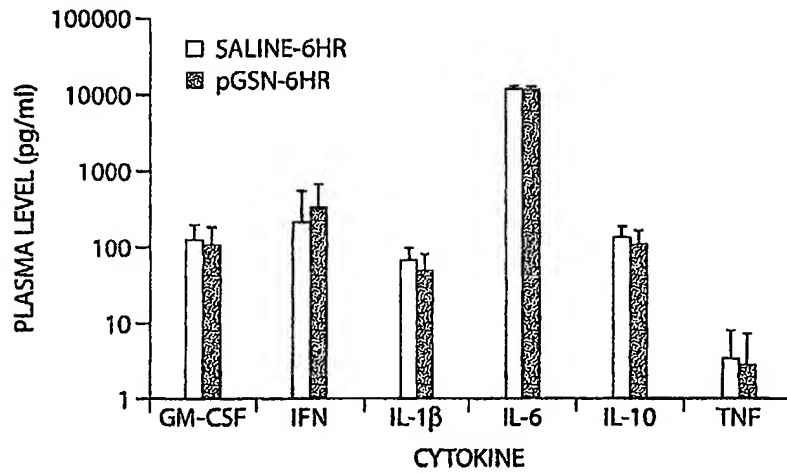


Fig. 4A

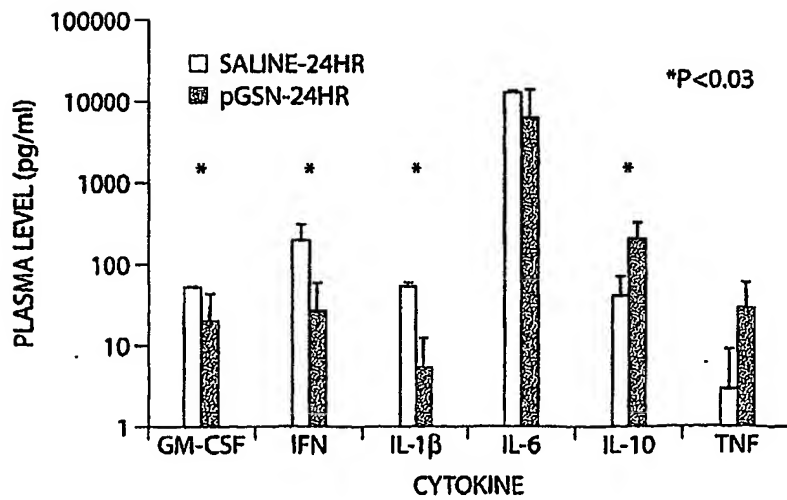


Fig. 4B

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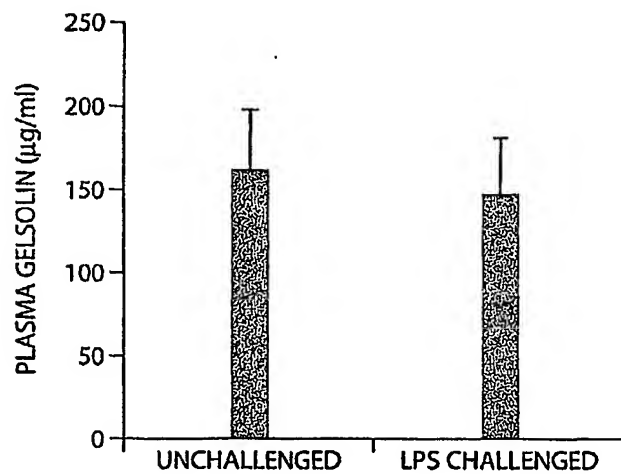


Fig. 5

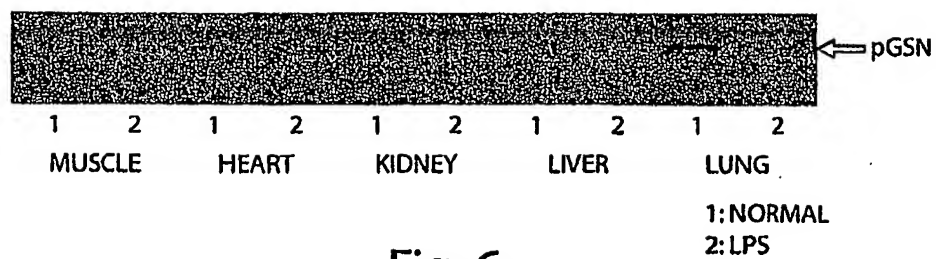


Fig. 6

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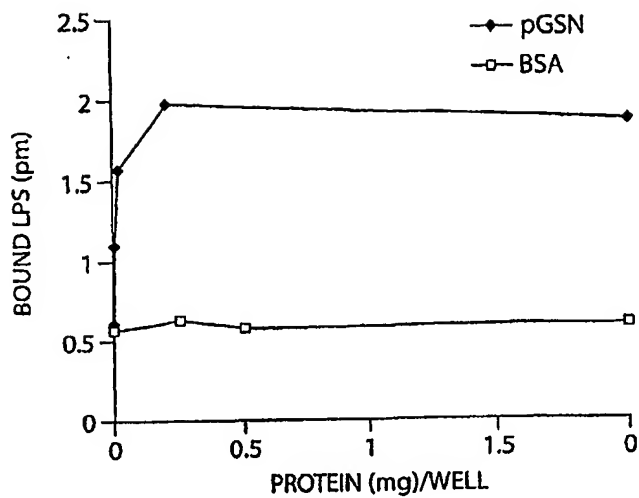


Fig. 7

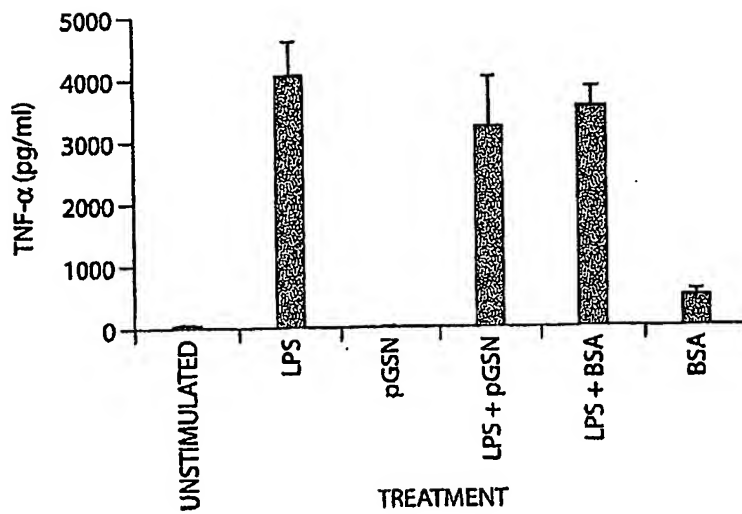


Fig. 8

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